

Review

What Defines a Plant Chemotype? Conceptual Boundaries, Chemical Criteria, and Methodological Challenges

Mauricio Mateus Guimaraes-Silva ^{1,†}, Matheus Chaves de Jesus ^{1,†}, Dannily Augusto Rebouças ¹ ,
Lorrayne Oliveira-Souza Abreu ^{1,2} , Claudete da Costa-Oliveira ¹ , Elaine de Lima de Jesus ¹ ,
Orlando Maia Barboza ¹ , Tamara do Nascimento da Silva ¹, Philippe Barreto de Almeida ¹,
Renato Crespo Pereira ³ , Davyson de Lima Moreira ²  and Ygor Jessé Ramos ^{1,*} 

¹ Farmácia da Terra Laboratory, Faculty of Pharmacy, Federal University of Bahia, Rua Barão de Jeremoabo, 147, Ondina, Salvador 40170-115, BA, Brazil; mauriciomsgs@gmail.com (M.M.G.-S.); matheuschavesj7@gmail.com (M.C.d.J.); dannily_rs@hotmail.com (D.A.R.); lorrayneoliveira@jbrj.gov.br (L.O.-S.A.); deteoliveira@hotmail.com (C.d.C.-O.); elainejesus.farmacia@gmail.com (E.d.L.d.J.); orlandomaiabarboza@gmail.com (O.M.B.); tmarasilva@gmail.com (T.d.N.d.S.); philippebarreto@gmail.com (P.B.d.A.)

² Research Institute of the Rio de Janeiro Botanical Garden, Rua Pacheco Leão 915, Jardim Botânico, Rio de Janeiro 22460-030, RJ, Brazil; davysonmoreira@jbrj.gov.br

³ Department of Marine Biology, Universidade Federal Fluminense, Campus do Valonguinho, Outeiro de São João Batista, s/n, Centro, Niterói 24020-141, RJ, Brazil; rcrespo@id.uff.br

* Correspondence: ygor.jesse@ufba.br

† These authors contributed equally to this work.

Abstract

Plant chemical diversity constitutes an essential dimension of biodiversity, arising from interactions among genetic background, biosynthetic regulation, the environment, ontogeny, ecology, and evolutionary history. Although the term chemotype is widely used for distinguishable chemical patterns within a taxon, its meaning remains inconsistent across plant biology, phytochemistry, pharmacognosy, chemical ecology, and natural-product standardization. This conceptual review examines the historical development, conceptual boundaries, chemical criteria, biological significance, and methodological requirements of plant chemotypes. We define a chemotype as a distinguishable, recurrent, relatively stable, analytically reproducible, and biologically interpretable intraspecific chemical pattern expressed by individuals, populations, or lineages of the same taxon. Assignment should be based primarily on chemical discontinuity, diagnostic markers, recurrence, relative stability, and analytical reproducibility; ecological, functional, biosynthetic, and genetic information should be treated as supporting evidence when available. The framework applies to volatile and non-volatile systems, including essential oils, cannabinoids, alkaloids, phenolics, and glucosinolates, while recognizing that platform coverage, annotation confidence, and taxon-specific biology can alter the inferred groups. We distinguish chemotypes from chemical phenotypes, chemical ecotypes, chemical geotypes, chronotypes, chemovars, and chemophenetic groups and propose three evidence levels: putative, supported, and confirmed chemotypes.

Keywords: chemophenetics; chemodiversity; specialized metabolites; chemical phenotype; plant plasticity; ecotype; geotype; chronotype; phytochemistry; chemical ecology



Academic Editor: Cheng Chang

Received: 12 June 2026

Revised: 28 July 2026

Accepted: 28 July 2026

Published: 30 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](#)

[Attribution \(CC BY\)](#) license.

1. Introduction

Plant chemical diversity constitutes a functional dimension of biodiversity, with implications for ecology, evolution, pharmacognosy, agriculture, domestication, biological conservation, and natural-product standardization [1,2]. Beyond taxonomic identity, plants express complex repertoires of specialized metabolites that are shaped by biosynthetic capacity, evolutionary history, and ecological context [3,4]. These metabolites modulate interactions with herbivores, pathogens, pollinators, mutualists, competitors, and abiotic factors [5,6], thereby influencing ecological performance, adaptation, and evolutionary trajectories across different levels of biological organization [7]. Accordingly, phytochemical diversity should be understood not merely as a catalog of compounds, but as a biochemical, functional, ecological, and evolutionary structuring attribute of plant life.

The recognition of chemodiversity as an organized biological phenomenon has broadened our understanding of the mechanisms that generate and maintain chemical variation in plants [8,9]. Such variation may arise from genetic differentiation, biosynthetic diversification, evolutionary convergence, phenotypic plasticity, ontogeny, environmental gradients, biotic interactions, and historical processes of domestication and selection [2,10–12]. Consequently, chemical profiles should be interpreted as dynamic components of the plant phenotype, capable of acting as niche dimensions and influencing ecological and adaptive responses [7]. Chemical variation should therefore not be assessed only in terms of the presence, absence, or relative abundance of compounds, but also in relation to biological stability, ecological role, genetic basis, temporal persistence, and methodological reproducibility [9,13].

In this context, the concept of chemotype has become relevant for designating distinguishable chemical patterns within the same species or within a clearly delimited taxonomic unit [13,14]. Chemotypes are often characterized by the predominance, absence, or differential proportion of one or more specialized metabolites belonging to a defined chemical family or biosynthetically related group [15]. However, a chemotype should not be inferred solely from the dominance of a compound when such dominance is not accompanied by evidence of chemical discontinuity, recurrence, relative stability, analytical reproducibility, or biological interpretation [16]. The term has been applied to different chemical systems, including essential oils and other classes of specialized metabolites. It has also been used in diverse scientific and applied contexts, including research on aromatic, medicinal, and toxic plants, plant breeding, pharmacognosy, and the authentication and standardization of plant-derived materials [15,17]. Such chemically distinct intraspecific groups may differ in ecological function, biological activity, agronomic value, toxicity, therapeutic potential, or industrial applicability [18,19]. In applied settings, chemotypic classification may support raw-material authentication, selection of elite genotypes, reproducibility of pharmacological assays, traceability of plant-derived products, and risk assessment in medicinal, food, and agricultural systems [11,19].

The scientific usefulness of the chemotype concept depends on the criteria used for its delimitation [14,16]. When well defined, a chemotype can reveal stable patterns of intraspecific chemical differentiation, contribute to chemophenetic or chemosystematic interpretations, and integrate phytochemical, ecological, genetic, and evolutionary evidence [18,20,21]. When used broadly or imprecisely, the term loses explanatory value and may obscure the mechanisms responsible for chemical variation [13]. This limitation is particularly relevant because the term has been applied heterogeneously in the botanical, phytochemical, ecological, pharmacognostic, and natural-product literature, sometimes to indicate relatively stable chemical lineages and sometimes merely to describe quantitative differences associated with the environment, plant organ, seasonality, ontogeny, extraction method, or sampling design [10,15,17].

This conceptual dilution has important methodological and interpretative consequences because operational classifications based solely on quantitative chemical differences may not correspond to biologically meaningful chemotypes. Distinguishing relatively stable intraspecific chemical patterns from environmentally or methodologically induced variation therefore requires explicit conceptual criteria, a robust sampling design, and appropriate analytical interpretation [10,13,15–17,22].

The distinction among genotype, chemotype, ecotype, and chemical phenotype is therefore central [16,23]. Genotype determines the biosynthetic potential of an organism, whereas the chemical phenotype corresponds to the complete metabolic profile expressed by an individual at a given time under the combined influence of genetic background, development, environmental conditions, and ecological interactions [2,8]. In contrast, a chemotype represents a recurrent and relatively stable subset of chemical phenotypes that shares consistent diagnostic chemical characteristics within a taxonomic unit. This distinction is essential because not every chemical phenotype reflects a stable chemotype: transient metabolic responses caused by environmental variation, ontogeny, or methodological factors may alter the chemical phenotype without representing a biologically meaningful intraspecific chemical group [7,9,16]. Consequently, a chemically distinct profile does not necessarily imply a genetically distinct lineage, just as genetically different individuals may converge chemically under similar selective pressures or environmental conditions [6,24]. Robust chemotype assignment therefore depends on integrating adequate sampling, analytical reproducibility, multivariate evidence, discriminant markers, and, whenever possible, biosynthetic or genetic support [21,25–27].

Against that background, this review examines the conceptual, chemical, biological, and methodological criteria required to define plant chemotypes and distinguish them from chemical phenotypes, chemical ecotypes, chemical geotypes, chemical chronotypes, and environmentally induced metabolic variation. We argue for the use of the term chemotype as a precise biological and methodological category, supported by chemical distinctiveness, stability, reproducibility, and functional or interpretative relevance [13,14,16].

2. Methodological Framework of the Review

Although this is a conceptual rather than a strict systematic review, an explicit search strategy was adopted to improve transparency and traceability. Scopus, Web of Science, and PubMed were searched using combinations of “chemotype”, “plant chemotype”, “chemical phenotype”, “chemophenetic”, “chemodiversity”, “ecotype”, “geotype”, “chronotype”, “plant specialized metabolites”, “metabolomic variation”, “essential oil chemotype”, and “chemical polymorphism”. Classical and recent literature were considered. The complete strategy, descriptors, and selection rationale for records published from 2021 to 2026 are provided in Supplementary Table S1.

Studies were included when they provided an explicit or operational definition of chemotype, analyzed intraspecific chemical variation, addressed volatile or non-volatile metabolites, related chemical composition to genetics, environment, ontogeny, seasonality, or taxonomy, or proposed statistical, chemometric, or metabolomic criteria for chemical differentiation. The selected evidence was synthesized to distinguish rigorous uses of the term chemotype from closely related categories, such as chemical phenotype, ecotype, chemical geotype, chemical chronotype, and metabolic variation induced by environmental or experimental factors.

Generative artificial intelligence was used as an auxiliary tool during manuscript preparation. Specifically, ChatGPT 5.5 (OpenAI, San Francisco, CA, USA) was employed to support scientific writing, language revision, textual organization, improvement of methodological wording, and refinement of analytical descriptions. Canva (Canva Pty

Ltd., Sydney, NSW, Australia) was used exclusively for the graphical design and layout of figures and schematic diagrams, without the use of artificial intelligence-based image generation or AI-assisted content creation. Artificial intelligence was not used to independently define the conceptual framework, select references, extract data, validate evidence, interpret results, or make methodological decisions. All AI-assisted outputs were critically reviewed, edited, and validated by the authors, who assume full responsibility for the originality, accuracy, integrity, interpretation, and final content of the manuscript.

3. Results and Discussion

3.1. Historical Development of the Chemotype Concept

3.1.1. Semantic Origin and Early Trajectory of the Term

The term chemotype derives from the association between the prefix “chemo-”, referring to the chemical composition or profile of substances produced by an organism, and “type”, understood as a recognizable category defined by distinctive characters. In a general sense, it may be understood as a “chemical type”, that is, a form of differentiation based on the chemical composition of individuals, populations, or lineages belonging to the same taxonomic group. However, its historical trajectory did not begin as a broad category of vascular plant phytochemistry, nor as a commercial designation for essential oils, but rather in a context closer to chemical systematics, lichenology, and the need to recognize morphologically similar organisms that displayed consistent chemical differences [14,20,28].

Before its consolidation in the study of aromatic and medicinal plants, related terms such as chemoform, chemotype, and chemovar were already circulating in fields such as microbiology, infraspecific nomenclature, and the classification of organisms differentiated by biochemical traits. In these contexts, such terms were used to distinguish lineages or groups based on chemical composition, the production of specific metabolites, or physiological attributes. However, the use of the suffix type required caution, because “type” has a specific nomenclatural meaning in taxonomic codes. For this reason, in certain microbiological and nomenclatural contexts, terms such as chemovar and chemoform came to be preferred when the intention was to indicate biochemical variation without assigning it the formal status of a taxonomic “type” [29,30].

The most frequently cited origin of the term chemotype, in an organismal and systematic sense, is attributed to Santesson [14], in the context of lichen taxonomy. In that work, the author used chemotype to designate chemically characterized fractions of a population composed of morphologically indistinguishable individuals. The proposal was directly connected to studies on “chemical races” in lichens, in which qualitative differences in the production of specific substances were used to recognize populations or intraspecific entities when conventional morphological characters were insufficient to distinguish them [14,28,31]. Thus, in its initial lichenological formulation, the chemotype referred to a relatively stable, recognizable, and potentially informative chemical discontinuity among morphologically similar organisms. However, morphological uniformity is not a universal requirement for recognizing chemotypes in vascular plants, which may occur with or without associated morphological, anatomical, physiological, or phenological differentiation. These associated traits should be interpreted as complementary characteristics rather than as defining criteria for chemotype assignment.

The consolidation of this approach was favored by the development and standardization of chromatographic methods, particularly thin-layer chromatography, which enabled the comparison of chemical profiles among taxa, populations, and herbarium specimens and promoted the incorporation of specialized metabolites into taxonomic studies [28,32]. This development coincided with the expansion of chemotaxonomy and chemosystemat-

ics during the second half of the twentieth century, in which the occurrence, absence, or predominance of metabolites was increasingly interpreted as evidence of taxonomic affinity, evolutionary history, and biosynthetic relationships [20,28,32–38]. Although chemical composition became an important diagnostic character, its taxonomic interpretation still required caution because intraspecific variation, environmental influence, and chemical convergence could produce similar phytochemical patterns [20,33]. Later, Gottlieb broadened this perspective by proposing micromolecular evolution as a conceptual framework linking secondary metabolism, ecology, and plant systematics [38].

In the field of essential oils, the perception that plants belonging to the same species can produce oils with distinct compositions predates the modern formalization of the term chemotype [39,40]. Since the traditional trade in aromatic raw materials, geographical provenance, ecological conditions, and cultivation environment have already been empirically recognized as factors capable of influencing the aroma, composition, and therapeutic or organoleptic properties of oils. By the late nineteenth and early twentieth centuries, many commercial essential oils were identified by designations associated with producing regions, reflecting the idea that territorial origin could determine specific chemical profiles. In this context, the notion of chemical geotype was used to indicate profiles associated with the provenance of plant raw material [39,40].

From the second half of the twentieth century onward, especially within the Francophone tradition of scientific aromatherapy and applied phytochemistry, the concept of chemotype became more widely disseminated in the study of aromatic plants. Pierre Franchomme contributed to popularizing the idea of the “biochemical signature” of plants, articulated through the terms chemotype, quimiotipo, 化学型, الكيمائي النمط, chimiotype, or race chimique [41,42]. This applied tradition linked predominant constituents to biological properties, therapeutic uses, and safety, but it also encouraged a simplification in which the chemotype was often reduced to the major essential oil constituent without considering the complete profile or its stability [41,43,44].

From the 1970s onward, chemotype became established in studies of aromatic and medicinal plants, particularly essential oil-producing species. *Thymus vulgaris* L. was a central model: French studies recognized recurrent “chemical types” or “chemical races” based on essential oil composition [45,46]. This transfer from lichen systematics to vascular plants established the ecological, pharmacognostic, therapeutic, and commercial relevance of intraspecific chemical differentiation [18,47].

In parallel, also during the 1970s, studies on *Cannabis sativa* contributed to broadening the application of chemotypic reasoning. Small and Beckstead [48] investigated different cannabinoid phenotypes in *Cannabis* stocks, demonstrating the existence of distinct chemical profiles based on the relative predominance of cannabinoids. Although the term chemotype was not always used explicitly in the titles or the initial formulation of these studies, this classification provided a conceptual basis for the later distinction among Δ^9 -tetrahydrocannabinol (THC)-rich, cannabidiol (CBD)-rich, and intermediate THC/CBD materials [48,49]. This contribution was particularly important because it demonstrated that intraspecific chemical differentiation could have direct consequences for the functional classification, medicinal use, regulation, and quality control of a species of major pharmacological relevance [25,50].

Between the late 1970s and the 1980s, studies of *T. vulgaris* progressed from describing different oils to interpreting chemotypes as expressions of chemical polymorphism. Natural populations in southern France showed recurrent profiles dominated by specific monoterpenes and associated with genetic differentiation and geographic structure. These patterns have been related to local adaptation and natural selection under varying abiotic conditions and biotic pressures, including herbivory [18,47,51]. This work established

the chemotype as a useful category for ecological and evolutionary inference, rather than merely for chemical identification.

In the 1990s and 2000s, the use of the term expanded into pharmacognosy, natural product phytochemistry, scientific aromatherapy, and the essential oil industry. Chemotype began to be applied to several aromatic plants, such as *Thymus*, *Ocimum*, *Lippia*, *Mentha*, *Rosmarinus/Salvia*, *Cinnamomum*, and others [20,52,53]. In this context, its function became increasingly operational: to guide the selection of plant raw materials, differentiate essential oils from the same species, predict biological properties, establish quality criteria, and support the standardization of extracts, herbal medicines, cosmetics, and aromatic inputs [43,54,55]. This applied diffusion, however, also brought conceptual simplifications, because the chemotype often came to be defined only by the major constituent of the essential oil, without adequately considering the full chemical profile, pattern stability, geographical origin, the plant part analyzed, or the environmental conditions of production [7,13].

From the 2000s onward, the limitations of major-compound classification became increasingly evident. Studies of *T. vulgaris* and other aromatic species incorporated broader quantitative profiles and multivariate statistics. Keefover-Ring et al. [13], for example, showed that classifications based on a few major compounds may obscure relevant intraspecific diversity. Chemotypes should therefore be treated as reproducible chemical groupings rather than as labels derived from the most abundant constituent of a single sample [7,13].

In the 2010s, the expansion of medicinal *Cannabis* reignited the debate on the adequacy of the terms used to classify plants and products with distinct chemical profiles. It became increasingly clear that commercial names, popular categories such as “sativa” and “indica”, and even the traditional notion of cultivar were not always sufficient to represent the actual chemical composition of plant materials. In this context, metabolomic studies began to advocate the use of the term chemovar to designate groups defined by reproducible profiles of cannabinoids, terpenes, and other relevant metabolites in *Cannabis* [50,56–58]. This shift had important implications for traceability, prescription, quality control, genotype selection, and the development of *Cannabis*-based medicinal products [50,58].

Also, from the 2010s onward, the chemotype gained greater relevance in regulatory, pharmacopoeial, and commercial contexts. In the essential oil and herbal medicine sectors, chemical identity came to be treated as a central element for the authentication, quality, and safety of raw materials. Technical standards, such as ISO 9235:2021, which addresses the vocabulary of natural aromatic raw materials, and ISO 11024-1:1998, which concerns the preparation and presentation of chromatographic profiles of essential oils, reinforced the importance of chemical profiling for product traceability and standardization [55,59]. Likewise, pharmacopoeial monographs came to use chromatographic profiles, marker limits, and chemical specifications as criteria for quality control and authentication of plant raw materials [54]. In these cases, the chemotype came to have a direct impact on commercial acceptance, product registration, and the definition of minimum quality standards [53,54].

An emblematic example of this application is *T. vulgaris*. Although the species presents different chemotypes, not all of them have the same pharmacopoeial or commercial status. European regulatory documents recognize the existence of chemical variation, but certain profiles, such as those rich in thymol and carvacrol, are more directly associated with the quality specifications required for *Thymi herba* [54,60]. This case demonstrates that the chemotype is not merely a descriptive category, but may determine the pharmacognostic value, therapeutic applicability, regulatory compliance, and commercial positioning of a plant raw material [53,60].

Since the 2020s, metabolomics, chemical fingerprinting, molecular traceability, chemical ecology, and plant breeding have further broadened the concept. Contemporary approaches may retain interpretable dominant markers while using complete profiles to represent intraspecific variation more accurately. This development is relevant to agronomic selection, domestication, conservation, authentication, quality control, and analyses of genotype–environment interactions [7,9,61,62].

This evolution also marks a transition from classical chemotaxonomy to chemophenetics. Chemophenetics examines chemical diversity across spatial, temporal, and organizational scales rather than treating metabolites only as taxonomic characters [7,9,61,62]. Within this framework, chemotype is one recurrent form of intraspecific chemical organization, not a synonym for every chemically separated group.

3.1.2. Historical Interpretations of the Chemotype Concept

Historical usage can be grouped into three interpretations: chemotype as a chemical race, as a recurrent chemical phenotype, and as an operational category. The first is closest to lichenology and chemosystematics and implies a relatively stable entity, ideally with hereditary support. *T. vulgaris* is the classical vascular-plant example because its essential oil polymorphism includes discrete patterns of monoterpene co-occurrence, geographic structure, and genetic control [13,18,63,64].

A second interpretation places chemotype within the broader chemical phenotype. Here, a chemotype is a recurrent and diagnostically meaningful pattern observed across comparable conditions, even when its complete genetic basis is unresolved. In *C. sativa*, cannabinoid ratios provide a strongly heritable foundation, but classification also depends on the markers and organs considered and may be refined by terpenoids and other metabolites [25,48,50,65–68].

Studies of *T. vulgare* further show that a chemotype explains only part of the total metabolomic variation and may interact with genotype, floral traits, herbivory, performance, and resource allocation [21,69]. The chemical phenotype is therefore the full metabolic state expressed at a given time, whereas the chemotype is a recurrent subset defined by stable diagnostic features [7,9].

The third interpretation is operational. In pharmacognosy, quality control, essential oil commerce, cultivar selection, medicinal *Cannabis*, and raw-material traceability, chemotype may denote a reproducible profile useful for identification, specification, or prediction of biological activity, even when genetic stability has not been demonstrated [15,50,54,59,67].

Operational use is legitimate provided that its evidential scope is explicit. The environment, organ, ontogeny, season, cultivation, post-harvest processing, extraction, and analytical platform can alter metabolite proportions [10,17]. A major-compound label may support commercial specification, but it cannot by itself justify evolutionary, taxonomic, or genetic inference.

The historical record therefore supports a restricted but flexible concept: chemotype should be reserved for chemical patterns with sufficient distinctiveness, recurrence, relative stability, analytical reproducibility, and interpretative relevance. This preserves both its biological meaning and its practical use while avoiding its reduction to a generic synonym for a chemical profile [7,9,16,62].

3.2. What Defines a Plant Chemotype?

A plant chemotype should be defined through a methodological design capable of distinguishing relatively stable intraspecific chemical patterns from circumstantial variation. The conceptual origin of the term is associated with the identification of chemically charac-

terized entities within morphologically indistinguishable populations, initially in lichenology and chemical systematics [14]. However, the mere observation of chemical variation within a species is not sufficient to recognize a chemotype. For a difference to be treated as chemotypic, it must constitute a robust, repeatable intraspecific pattern supported by chemical, biological, and methodological criteria. This perspective is consistent with the notion that chemotypes correspond to intraspecific chemical variations in which different individuals or specimens of the same species produce distinct types, proportions, or combinations of specialized metabolites under genetic and environmental conditioning [70]. The chemophenetic and chemodiversity approach further reinforces that this variation should be interpreted across spatial, temporal, and organizational scales, avoiding the reduction in the chemical phenomenon to a rigid taxonomic classification or to an isolated major marker [62].

The first requirement for defining a chemotype is the existence of intraspecific chemical discontinuity or consistent multivariate separation. This discontinuity may involve the presence or absence of compounds, the predominance of a given metabolite, a stable shift in chemical ratios, the occurrence of diagnostic biosynthetic groups, or the formation of discriminant metabolomic profiles. However, the difference must be sufficiently marked to separate chemical groups within the species and should not merely reflect gradual, transient, or methodologically unstable quantitative fluctuations. Studies on the intraspecific diversity of specialized metabolites show that chemical variation may be maintained by ecological, genetic, and evolutionary processes, but may also result from phenotypic plasticity, biotic interactions, environmental gradients, and spatial heterogeneity [6,8,9]. Consequently, chemotypic discontinuity should be interpreted within the ecological and biological context of the species, because not every detectable difference in a chemical matrix represents a chemotypic difference.

The second requirement involves dominant or diagnostic chemical markers. Historically, many chemotypes have been defined by the major compound, especially in essential oils. This strategy may be useful in applied contexts such as quality control, scientific aromatherapy, pharmacognosy, and the selection of raw materials, but it is insufficient when applied in isolation. In *T. vulgaris*, for example, chemotypes were associated with predominant monoterpenes, but subsequent studies showed that differentiation among groups involves qualitative and quantitative patterns of co-occurrence, rather than a single main constituent [13,63]. Thus, the chemotypic marker may be a major compound, a metabolic ratio, a chemical class, a biosynthetic signature, or a multivariate set of metabolites.

This broader perspective is particularly important in complex chemical matrices. In *Cannabis sativa*, classifications based only on major cannabinoids do not capture the full chemical diversity of the species, because terpenoid profiles, organ-specific markers, and other specialized metabolites may discriminate additional categories [71,72]. In *Tanacetum vulgare* L. (Asteraceae), metabolomic fingerprints retain the ability to recognize previously described chemotypes but reveal broader chemical variation than that captured by dominant terpenes alone [21]. From a statistical standpoint, this approach requires appropriate treatment of compositional data, because relative abundances, proportions, and closed sums may produce biased interpretations when analyzed using inadequate methods [73]. Thus, chemotype definition should combine interpretable markers with chemometric, metabolomic, and biosynthetic approaches capable of representing the complexity of the chemical profile.

The third requirement is reproducibility across individuals, populations, and sampling events. A chemotype should not be inferred from a single plant, a single collection event, a single plant organ, or a single analytical condition. The production of specialized metabolites is sensitive to environmental, physiological, and methodological factors, in-

cluding light availability, temperature, water availability, nutrients, herbivory, pathogens, seasonality, phenology, plant age, the organ analyzed, post-harvest processing, extraction method, and analytical platform [10,17,74]. Without replicated sampling and analytical standardization, an apparent chemical grouping may reflect organ-specific expression, environmental plasticity, or methodological dependence rather than a stable intraspecific pattern. In *Tanacetum vulgare*, root terpenoid profiles, dominated primarily by sesquiterpenoids, were compositionally distinct from the monoterpenoid-dominated leaf profiles and did not reproduce the leaf-based chemotype clusters [75]. In *Thymus vulgaris*, ethanol extraction revealed an additional chemotype that had not been recognized in conventional essential oil classifications, showing that extraction strategy can alter the inferred number and boundaries of chemotypes [13]. Comparisons between wild specimens and successive generations of monoclonal *Piper gaudichaudianum* and *P. mollicomum* cultivars likewise demonstrated substantial chemical phenoplasticity under cultivation [76]. These examples do not invalidate the observed chemical differences, but they demonstrate that chemotype assignment must explicitly specify the plant organ, chemical family, sampling context, extraction procedure, and analytical method.

The fourth requirement is relative stability under controlled or comparable conditions. Chemotypic stability does not imply absolute invariability, because every plant chemical profile may vary to some extent. Rather, it means that the chemical pattern remains recognizable when the main sources of variation are controlled, described, or adequately compared. The classic case of *T. vulgaris* demonstrates that essential oil polymorphism may show genetic control and relatively stable chemical patterns [18,63]. In *Cannabis sativa*, the inheritance of the chemical phenotype has been experimentally demonstrated, although final expression depends on markers, tissues, and cultivation conditions [66]. In *Piper*, the comparison between wild specimens and monoclonal cultivars from different generations indicated that part of the variation in essential oils may result from chemical phenotypic plasticity, highlighting the need to separate chemical stability, environmental response, and phenotypic expression [76]. Thus, stability should be treated as relative, experimentally assessable, and dependent on sampling design.

Supporting biological evidence strengthens chemotype interpretation but should not be treated as an obligatory fifth requirement. Such evidence may include coherence with plausible biosynthetic routes, chemical families, metabolic fluxes, enzymes, functional modules, ecological pressures, or genetic mechanisms. Plant chemical diversity results from the evolution of biosynthetic pathways, enzymatic specialization, gene duplication and reorganization, metabolic convergence, and the emergence of compounds with ecological and adaptive functions [2,3,77–79]. Biological coherence therefore helps distinguish biologically interpretable groups from purely statistical partitions, but the absence of genetic or biosynthetic confirmation does not invalidate a recurrent, relatively stable, and analytically reproducible chemotype.

Thus, a plant chemotype can be defined as an intraspecific chemical pattern characterized by robust chemical discontinuity or consistent multivariate separation, diagnostic markers in explicitly defined plant part(s), organ(s), or tissue(s), recurrence across individuals and sampling events, relative stability under controlled or comparable conditions, and analytical reproducibility. Biosynthetic, ecological, functional, or genetic coherence increases the level of support but is not mandatory for initial or operational chemotype assignment. The absence of the core criteria does not eliminate the relevance of the observed chemical variation, but it calls for terminological caution. In such cases, it may be more appropriate to use categories such as chemical phenotype, chemical ecotype, chemical geotype, chemical chronotype, or contextual metabolomic variation until additional evidence supports the existence of a chemotype *sensu stricto* [15,16]. The conceptual re-

relationships among chemotype, chemical phenotype, chemical ecotype, chemical geotype, chemical chronotype, chemovar, and chemodiversity are summarized in Figure 1. The logical sequence of criteria used for chemotype assignment is represented in Figure 2.

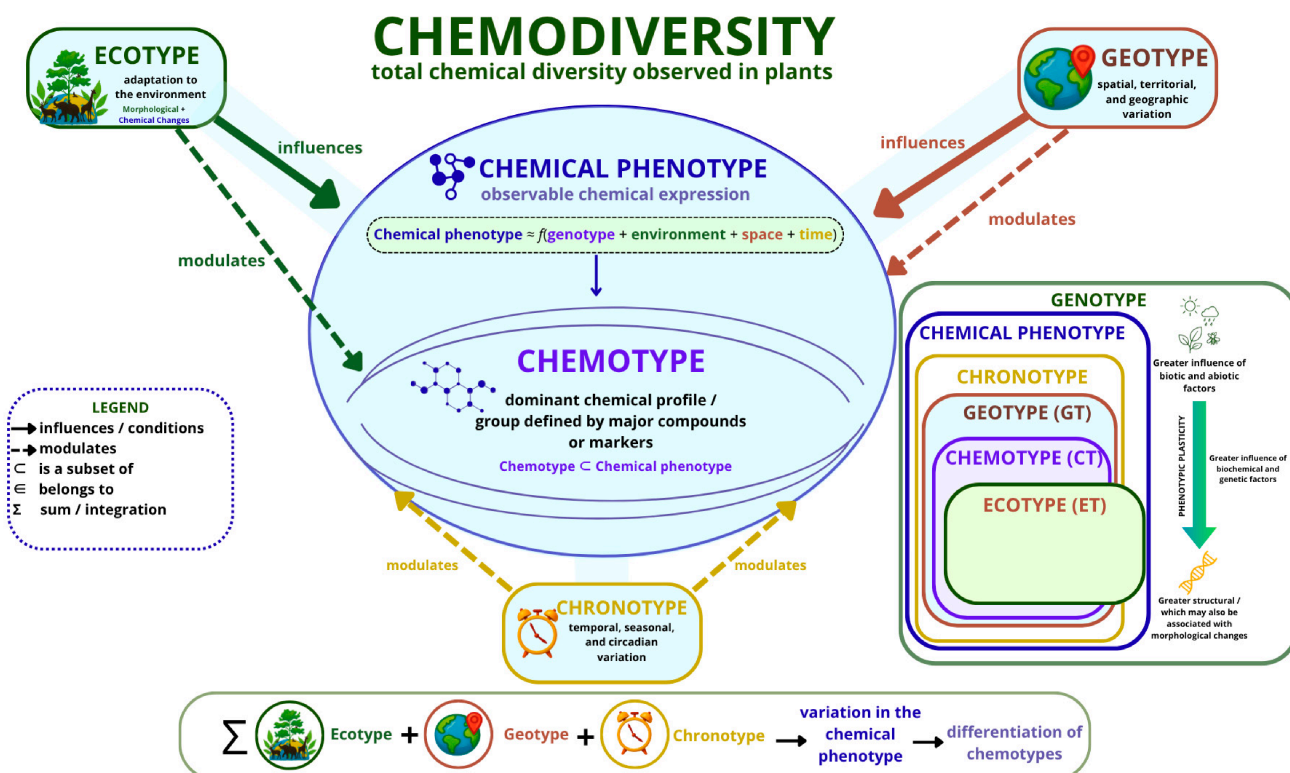


Figure 1. Conceptual relationships among plant chemotype, chemical phenotype, chemical ecotype, chemical geotype, chemical chronotype, chemovar, chemophenetic groups, and chemodiversity.

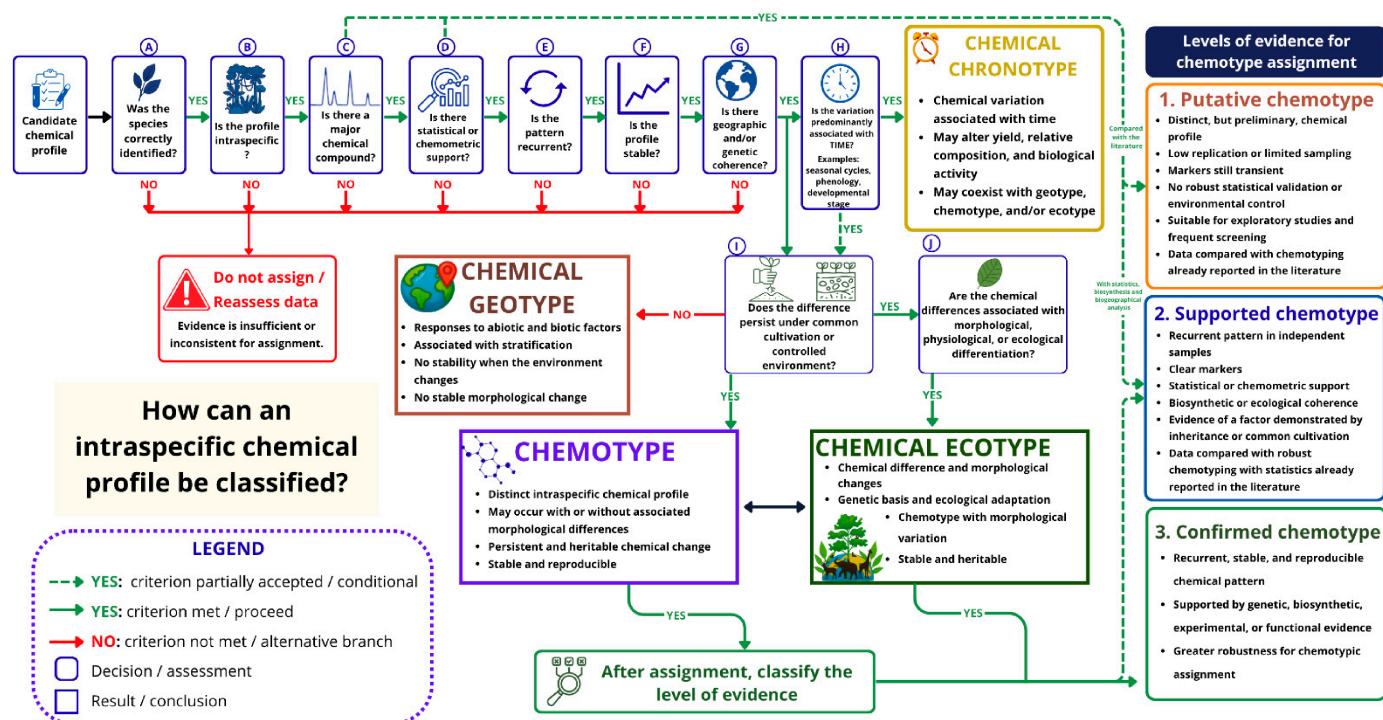


Figure 2. Decision tree and levels of evidence for plant chemotype assignment.

3.3. Conceptual Boundaries: What Is Not Necessarily a Chemotype?

A rigorous delimitation of the chemotype concept requires distinguishing it from closely related categories, especially chemical phenotype, ecotype, chemical geotype, chemical chronotype, and chemovar (Figure 1). These categories do not constitute lower or higher hierarchical levels, but rather express different causal, temporal, spatial, ecological, methodological, and applied scales of intraspecific chemical variation. The absence of this distinction favors the imprecise use of the term chemotype as a generic synonym for any difference in chemical composition observed among individuals, populations, organs, localities, seasons, or cultivated materials [62,70,76].

The chemical phenotype corresponds to the metabolic profile effectively expressed by a plant in each genetic, physiological, ontogenetic, environmental, temporal, and methodological context. It is a dynamic expression of specialized metabolism, resulting from the interaction among genetic potential, physiological state, and environmental conditions. Analytical procedures do not modify the phenotype itself, but determine how it is detected, quantified, and interpreted. In this sense, every chemotype, geotype, ecotype, or chronotype may be understood as a particular form of a recurrent, distinguishable, and relatively stable chemical phenotype; however, not every chemical phenotype should be interpreted as a chemotype. This distinction is fundamental because phenotypic plasticity is a central property of plants, allowing morphological, physiological, and metabolic adjustments in response to environmental heterogeneity (Figure 1) [23,24].

Chemical changes induced by drought, light availability, nutrients, herbivory, pathogens, soil, seasonality, phenological stage, plant organ, plant age, management practices, or extraction method may produce distinct profiles without constituting chemotypic categories. Müller and Junker [7] proposed that the chemical phenotype should be understood as a dynamic niche dimension, that is, as a functional dimension of the interaction between the plant and the environment. This formulation broadens the ecological relevance of chemical variation, but reinforces the need not to confuse circumstantial, transient, or organ-specific expressions with stable chemotypes. The chemophenetic approach recognizes that specialized metabolites vary across different spatial, temporal, and organizational scales, requiring careful control and interpretation of these sources of variation before stable categories such as chemotypes are proposed [62,70,76]. A concrete example is provided by *Piper gaudichaudianum* and *P. mollicomum*: chemical groupings observed in wild specimens were not consistently retained in bi-generational monoclonal cultivars under common cultivation, indicating that part of the apparent separation reflected cultivation-dependent phenoplasticity rather than stable chemotypic differentiation [76].

3.3.1. Chemical Ecotype

The distinction between chemotype and ecotype is also indispensable. The concept of ecotype refers to intraspecific variants adapted to environmental conditions, generally involving genetic differentiation associated with local adaptation and potentially including morphological, physiological, phenological, ecological, and chemical differences [80–83]. A chemical ecotype may exhibit differentiated chemical composition, but it is not defined exclusively by it. Thus, a chemically distinct population occurring in each environment should not automatically be classified as a chemotype without adequate sampling control, comparison among environments, assessment of recurrence, analysis of stability, and, whenever possible, genetic evidence or common-garden experiments capable of separating environmental plasticity from stable biological differentiation (Figure 2).

A well-documented chemical-ecotype example is *Arabidopsis halleri* along Alpine elevation gradients. Populations maintained under prolonged common-garden conditions retained differences in glucosinolate composition: high-elevation populations showed

higher relative proportions of indole glucosinolates, and these profiles were associated with lower field herbivory and altered oviposition preference by *Pieris brassicae* [84]. Because the chemical differences persisted under common conditions and were linked to an ecological gradient and herbivore interactions, this case supports the interpretation of chemical ecotypes rather than transient environmental phenotypes.

Whereas the ecotype emphasizes local adaptation and ecological differentiation, the chemotype emphasizes recurrent, distinguishable, and biologically interpretable intraspecific chemical patterns. The two categories may overlap, for example when a population adapted to a particular environment exhibits a stable and reproducible metabolic profile. However, this overlap does not justify treating them as strict synonyms. In our conceptual framework, ecotypes are understood as variants adapted to specific environmental conditions, whereas chemotypes correspond to intraspecific chemical variation in the types or proportions of specialized metabolites, conditioned by genetic, environmental, and historical-evolutionary factors [70]. This distinction preserves the analytical specificity of each category.

3.3.2. Chemical Geotype

A chemical geotype refers to a compositional pattern associated with geographic origin, territorial provenance, or the environmental and biocultural conditions characterizing a particular locality. In aromatic and medicinal plants, altitude, temperature, precipitation, light exposure, water availability, soil properties, associated microbiota, biotic interactions, cultivation practices, harvesting traditions, and local processing methods may collectively shape the chemical profile. A chemical geotype therefore expresses an association between the chemical phenotype and a geographically structured environment, which may include both ecological conditions and historically established biocultural practices. It does not necessarily represent an intrinsically stable or genetically fixed chemical lineage [10,17,64,70,74].

Geographic origin alone, however, does not imply chemotypic stability. A population may exhibit a characteristic chemical composition in its native environment and subsequently modify that profile when transferred to another region or subjected to different cultivation, harvesting, or processing conditions. The magnitude of this response depends on phenotypic plasticity, environmental variation, management practices, and the relative contribution of genetic factors. By contrast, a chemotype represents a recurrent, consistent, and reproducible intraspecific chemical variant whose differentiation cannot be explained solely by geographic provenance or by a local environmental response. A regional chemical profile should therefore not be classified as a chemotype unless its recurrence and stability have been demonstrated across independent samples, production cycles, or contrasting environmental conditions [64,70].

In commercial contexts, geographic and territorial names may legitimately be used to differentiate raw materials, essential oils, extracts, and other plant-derived products by communicating provenance, traceability, authenticity, cultural identity, traditional knowledge, or territorial reputation. Such information may contribute to the biocultural and economic valorization of a product, particularly when its identity is linked to local production systems, community practices, traditional processing methods, and the ecological characteristics of the territory. Nevertheless, commercial expressions such as “oil from region X”, “regional type”, “territorial origin”, “Geotype (GT)” or “product from a specific provenance” primarily constitute origin-related or territorial designations and do not, by themselves, demonstrate the existence of either a chemotype or a chemical geotype.

When the purpose is to emphasize the commercial, cultural, and territorial identity of a product without implying scientifically demonstrated chemical differentiation,

terms such as territorial provenance designation, biocultural-territorial designation, origin-linked product identity, or territorial product identity may be more appropriate. The expression geographical indication should be reserved for products protected under the applicable legal and regulatory framework. When a distinctive chemical composition has been demonstrated to be associated with a particular locality but remains predominantly dependent on environmental or management conditions, the terms chemical geotype, regional chemical profile, or geographic variation in the chemical phenotype are preferable. The term chemotype should remain restricted to intraspecific chemical patterns supported by evidence of recurrence, stability, and reproducibility beyond their environment of origin (Figure 2) [64,70]. A nominal example is *Origanum vulgare* cultivated in different European countries, in which essential oil yield, composition, and the frequency of thymol- and carvacrol-dominant profiles varied among provenances and cultivation environments [85]. Unless such differences persist after reciprocal or common-garden testing, they are more appropriately reported as chemical geotypes or regional chemical profiles.

3.3.3. Chemical Chronotype

Chemical chronotype refers to time-related metabolic patterns, including circadian, seasonal, phenological, ontogenetic, or interannual variation. This concept is particularly relevant in studies of essential oils, in which yield, relative composition, proportions among chemical classes, and biological activity may vary throughout the day, between dry and rainy periods, across reproductive stages, or during plant development (Figure 2). Studies on *Piper mollicomum* Kunth. (Piperaceae) demonstrated changes in essential oil composition among different vegetative parts and throughout the circadian rhythm [86], whereas analyses of *Piper gaudichaudianum* Kunth. (Piperaceae) explored seasonal and circadian variation using a chemophenetic approach and a reduction–oxidation index [87]. Studies on *Pectis brevipedunculata* Sch. Bip. and *Chromolaena corymbosa* (Aubl.) R.M.King & H.Rob. also indicate that seasonality and circadian rhythm can alter essential oil composition and bioactivity [88,89].

These temporal patterns are biologically relevant because they reveal the dynamic nature of specialized metabolism and its sensitivity to physiological, phenological, and environmental rhythms over time. However, they should not automatically be designated as chemotypes. Circadian, seasonal, or ontogenetic variation may represent the temporal expression of the chemical phenotype rather than a stable chemotypic category. For a temporal difference to be interpreted as a chemotype, it is necessary to demonstrate that the chemical pattern remains distinguishable and recurrent after controlling for the relevant temporal variables. Otherwise, the more appropriate designation is chemical chronotype, seasonal chemical profile, circadian chemical phenotype, or ontogenetic variation in specialized metabolism [7,62,70,76].

3.3.4. Chemovar

The distinction between chemotype and chemovar deserves particular attention, especially in *C. sativa*. The term chemovar has been used in agronomic, commercial, pharmaceutical, and regulatory contexts to classify cultivated materials based on chemical profiles, particularly cannabinoid and terpenoid profiles [50,67,68,71]. The term chemotype has a broader use in chemical ecology, chemosystematics, chemophenetics, pharmacognosy, evolutionary biology, and studies of intraspecific variation. In *Cannabis*, classical classifications based on cannabinoid phenotypes have demonstrated relevant differences among stocks, lineages, and cultivated materials [48,66], whereas recent metabolomic approaches have shown that multivariate profiles can discriminate materials more robustly than categories based on a few marker metabolites [72].

Thus, chemovar and chemotype may overlap, especially when cultivated materials exhibit recurrent, standardized, and biologically interpretable chemical profiles. However, they should not be treated as automatic synonyms. The notion of chemovar emphasizes cultivated material, traceability, standardization, agronomic identity, pharmaceutical purpose, or regulatory use. Chemotype, in contrast, should retain a broader biological and methodological definition, linked to distinguishable, recurrent, relatively stable, analytically reproducible, and biologically coherent intraspecific chemical patterns. In other words, a chemovar may have a chemotypic basis when supported by robust chemical data, but not every chemotype corresponds to a chemovar, especially in natural populations, ecological variation, or chemosystematic patterns not linked to cultivated materials [7,62,70,76].

3.3.5. Conceptual Boundaries

Table 1 summarizes the conceptual boundaries and defining criteria that distinguish the chemotype from related categories discussed in the previous sections.

Table 1. Definitions and conceptual boundaries of plant chemical types.

Chemical Type	Proposed Definition	Main Defining Axis	Conceptual Boundary
Chemotype	An intraspecific chemical pattern that is distinguishable, recurrent, relatively stable, analytically reproducible and biologically interpretable, expressed by individuals, populations or lineages belonging to the same taxon.	Chemical discontinuity; dominant or diagnostic markers; recurrence; relative stability; and analytical reproducibility. Biosynthetic, ecological, functional, or genetic evidence provides additional support when available.	It should not be defined only by the major compound or by circumstantial quantitative variation. Differences resulting exclusively from organ-specific sampling, seasonal variation, collection time, extraction method, or analytical artifacts should not be interpreted as distinct chemotypes unless chemotype assignment is consistently based on the same plant organ and supported by evidence of recurrence and stability.
Chemovar	A cultivated, selected or standardized plant material classified according to its chemical profile, especially in agronomic, pharmaceutical, commercial or regulatory contexts.	Cultivation, standardization, traceability, intended use and reproducible chemical identity of a plant material.	It may contain a chemotypic basis, but it is not automatically synonymous with a chemotype. The term is more appropriate for cultivated or regulated materials than for natural populations.
Chemical Ecotype	An intraspecific variant adapted to certain environmental conditions, potentially involving morphological, physiological, phenological, ecological and chemical differences.	Local adaptation and ecological differentiation.	A chemically distinct population in a given environment is not necessarily a chemotype. Ecotype and chemotype may overlap only when the chemical pattern is recurrent, stable and biologically supported.
Chemical Geotype	A chemical pattern associated with geographical origin, territorial provenance or local environmental filters such as altitude, soil, climate, latitude, longitude or regional management.	Spatial origin and territoriality (biocultural and geographical structuring) of chemical variation.	It describes geographical structuring of chemical profiles but does not necessarily indicate a stable chemotype independent of local environmental conditions.
Chemical Chronotype	A chemical pattern related to time, including circadian, seasonal, phenological, ontogenetic or interannual variation.	Temporal modulation of specialized metabolism.	It should be used when chemical variation is primarily time-dependent. A seasonal or circadian profile should not be named a chemotype unless stability and recurrence remain after temporal variables are controlled.
Chemical Phenotype	The metabolic profile expressed by a plant under a given genetic, physiological, ontogenetic, environmental, and temporal context.	Dynamic expression of specialized metabolism.	Every chemotype is a particular form of a recurrent and relatively stable chemical phenotype, but not every chemical phenotype is a chemotype.

Table 1. Cont.

Chemical Type	Proposed Definition	Main Defining Axis	Conceptual Boundary
Chemophenetic group	A group defined by chemical similarities or fingerprints interpreted through chemophenetic analysis across spatial, temporal and organizational scales.	Chemical similarity, metabolomic structure, chemodiversity and multivariate grouping.	It is an analytical group and should not be treated automatically as a taxonomic unit or a chemotype unless it satisfies criteria of discontinuity, recurrence, relative stability, and analytical reproducibility; biological interpretation provides additional support when available.

3.4. Methodological Criteria for Defining Plant Chemotypes

The definition of plant chemotypes requires a methodological design capable of distinguishing relatively stable intraspecific chemical patterns from circumstantial variation produced by the environment, ontogeny, phenology, plant organ, time of collection, season, sample processing, or analytical platform, as schematized in Figure 3. Thus, the proposal of a chemotype should not rely solely on the detection of chromatographic or metabolomic differences, but on a methodological sequence that includes representative sampling, botanical authentication, environmental metadata, analytical standardization, reliable metabolite identification, and adequate statistical support. This requirement is consistent with a chemophenetic approach, which interprets specialized-metabolite diversity across spatial, temporal, and organizational scales, rather than as the simple product of taxonomic differences or isolated chemical markers [62].

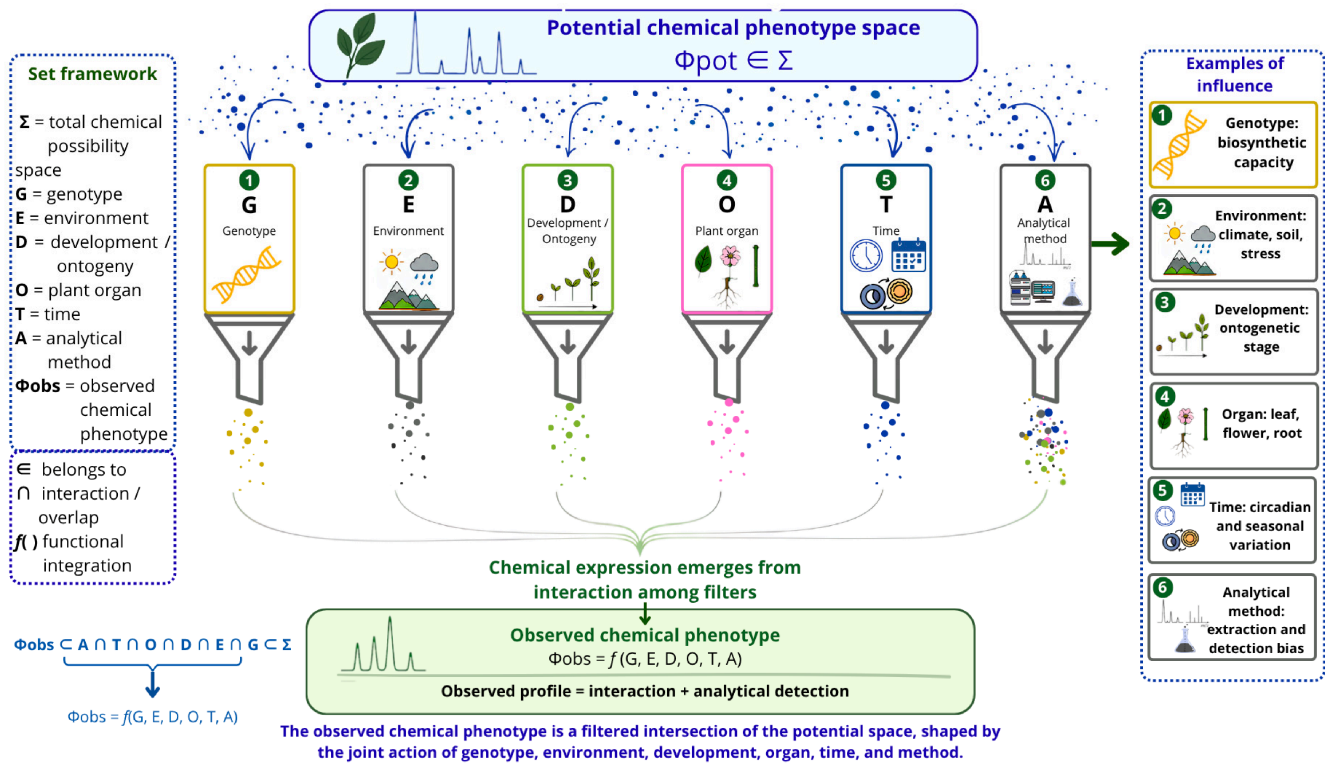


Figure 3. Biological determinants, analytical representation, and criteria for plant chemotype assignment.

The literature does not define chemotype through a single universal calculation procedure. What is observed is the coexistence of different delimitation strategies, which vary according to the chemical matrix, the objective of the study, the biological scale, and the degree of evidence available. Operationally, chemotypes have been recognized through four main routes: predominance of major compounds, presence or absence of diagnostic

markers, proportions or ratios among relevant metabolites, and multivariate separation of complete chemical profiles. Thus, calculation can be useful for standardizing nomenclature, but it does not replace the biological interpretation of the chemical pattern, including its ecological, functional, biosynthetic, and genetic context. The central difference is that a numerical rule allows chemical groups to be named transparently, whereas stronger chemotype assignment requires recurrence, relative stability, analytical reproducibility, and robust chemical validation; biosynthetic, ecological, functional, or genetic coherence provides supporting evidence when available [7,9,13–15,18]. In general, four criteria are most recurrent:

(1) *Predominance of major compounds*

This criterion is used mainly in studies of essential oils, in which the chemotype is named after the dominant constituent, such as thymol, carvacrol, linalool, geraniol, camphor, or 1,8-cineole. This model is classical in *T. vulgaris*, whose chemotypes have been related to discrete chemical patterns, geographical distribution, and partial or demonstrated genetic control [13,18,46,63].

(2) *Qualitative presence or absence of diagnostic markers*

This criterion applies when metabolites or biosynthetic products are consistently present in one chemical group and absent from another. In *Arabidopsis thaliana*, natural variation at the AOP2 and AOP3 loci generates qualitatively distinct glucosinolate profiles: functional AOP2 produces alkenyl glucosinolates, functional AOP3 produces hydroxyalkyl glucosinolates, and accessions lacking both functional enzymes accumulate methylsulfinylalkyl precursors [90]. This type of mutually exclusive biosynthetic product provides a genuinely qualitative marker and should be distinguished from classifications based on relative abundance or ratios [25,48,50,65,66,72].

(3) *Metabolic ratios or quantitative dominance criteria*

Some studies adopt percentage thresholds or relationships among compounds to make classification more transparent. One example is the key proposed by De Medeiros et al. [91], in which chemotypes are classified as A, A/B, or A/B/C according to the relative dominance of the major constituents. Thus, a sample can be classified as chemotype A when a single compound clearly dominates the profile (>51% of the total extract); as A/B when two compounds (>51%) show hierarchically organized chemical relevance; or as A/B/C when three compounds (>51%) structure the chemical profile. When these criteria are not met, the sample should be treated as a mixed or undefined profile. This operational approach is conceptually consistent with the distinction between pure and mixed chemotypes proposed by Holopainen and co-workers [92], in which some individuals exhibit a clearly dominant biosynthetic profile whereas others display intermediate or mixed chemical characteristics. This type of criterion is useful for standardization, but should be interpreted as an operational rule, not as automatic confirmation of a stable chemical lineage.

(4) *Multivariate separation of chemical profiles*

This criterion is used when differentiation does not depend on a single compound, but on a set of metabolites. Multivariate analyses and chemometric approaches can identify robust chemical groupings when supported by appropriate statistical validation and biological interpretation. Studies on *Tanacetum vulgare* indicate that leaf terpenoid chemotypes can be recognized by dominant markers, whereas overall metabolomic variation reflects the combined effects of genotype, environment, herbivory, and plant performance [21,27,69]. Robustness should be assessed by comparing alternative clustering algorithms, linkage criteria, distance metrics, preprocessing choices, and cluster-validity

indices; a grouping should not be accepted when it appears only under a single arbitrary analytical specification.

3.4.1. Sampling Design

Sampling design is the first requirement for biologically meaningful chemotype classification. Studies should include representative numbers of individuals, populations, and biological replicates while documenting the plant organ analyzed, developmental stage, collection conditions, and environmental metadata. Standardized sample preparation, quality control, and analytical reproducibility are likewise essential, particularly for specialized metabolites whose composition is strongly influenced by biological and environmental variation [10,17,74,93–96]. Failure to control these sources of variation may lead to the erroneous recognition of localized or transient chemical profiles as distinct chemotypes. Studies on *Piper mollicomum*, *Piper gaudichaudianum*, *Pectis brevipedunculata*, and *Chromolaena corymbosa* illustrate how organ-specific, seasonal, and temporal variation can alter chemical composition, emphasizing that such factors must be controlled or explicitly incorporated into the analytical design [86–89].

3.4.2. Botanical Authentication

No chemotype should be proposed without rigorous botanical authentication. The taxonomic identity of the sample should be confirmed by a specialist and accompanied by a voucher specimen deposited in a recognized herbarium, a voucher number, traceability of provenance, and updated scientific nomenclature. In research involving medicinal plants, natural products, and plant materials for pharmaceutical use, the absence of a voucher compromises verifiability, reproducibility, and the possibility of future reinterpretation of the results [97–100]. When necessary, authentication should be complemented by morphoanatomical characters, molecular markers, photographic documentation, tissue samples for genomic studies, and pharmacognostic criteria [101–104].

Botanical authentication is especially relevant because the chemotype operates within a previously defined taxonomic unit. If species identity is incorrect, the supposed intraspecific variation may instead represent a species mixture, unresolved synonymy, misidentification, hybridization, or interspecific variation. Thus, chemotypic classification depends on a basic premise: the individuals compared must belong to the same taxon or to a clearly delimited taxonomic unit. Without this condition, the chemotype ceases to be an intraspecific category and instead reflects taxonomic uncertainty [105]. Whenever available, molecular markers and population genetic data provide complementary evidence to confirm taxonomic identity and help distinguish stable genetically structured chemotypes from environmentally induced chemical variation, thereby strengthening the biological interpretation of chemotypic classifications.

3.4.3. Environmental Metadata

The interpretation of plant chemotypes requires the systematic recording of environmental metadata. Soil, altitude, latitude, longitude, climate, temperature, precipitation, humidity, light availability, water availability, cultivation regime, abiotic stress, biotic interactions, agronomic management, and collection history should be described whenever possible. The Metabolomics Standards Initiative emphasizes that the environmental context of biological samples should be reported in a minimal and standardized manner, because the absence of this information limits the interpretation and comparability of metabolomic data [106,107]. In plants, this requirement is fundamental because the chemical phenotype constitutes a dynamic niche dimension, modulated by interactions among genotype, physiology, and the environment [7].

The literature on specialized metabolites demonstrates that exogenous factors can produce substantial changes in chemical profiles, especially in aromatic, medicinal, and essential oil-producing species [10,17,74]. The chemical biogeography of widely distributed species also shows that spatial patterns may coexist with temporal variation, making it difficult to distinguish among chemotype, chemical geotype, and environmentally induced chemical phenotype [64]. Biotic, abiotic, genetic, biochemical, and anthropogenic factors should therefore be treated as integrated dimensions that modulate the chemical composition of medicinal and aromatic plants.

Although these categories may overlap under certain biological conditions, distinguishing them remains essential because they address different biological dimensions of chemical variation. Chemotypes describe relatively stable intraspecific chemical patterns, whereas chemical phenotypes encompass the complete metabolic expression of an individual, including transient responses to environmental, developmental, or methodological factors. Maintaining this distinction prevents temporary metabolic variation from being misinterpreted as stable intraspecific chemical differentiation and improves biological interpretation, reproducibility, and comparability among studies.

3.4.4. Analytical Standardization

Analytical standardization is indispensable because different chemical platforms do not capture the same fraction of chemodiversity. GC-MS, GC-FID, LC-MS/MS, UHPLC-HRMS, NMR, and targeted or untargeted metabolomics each have their own capabilities, limitations, and biases. In essential oils, constituent identification requires, at a minimum, comparison of mass spectra, retention indices, homologous alkane series, and specialized libraries, following the established tradition of gas chromatography and mass spectrometry [108–111]. Standards such as ISO 11024-1:1998 and ISO 9235:2021 reinforce the importance of chromatographic profiles and standardized terminology for natural aromatic raw materials [55,59].

For quantitative analyses, the use of appropriate internal or external standards should be explicitly reported. Whenever accurate quantification is required, detector response factors should be taken into account, since different metabolites may generate different analytical responses under identical instrumental conditions. Calibration curves and validated quantitative procedures improve the accuracy, reproducibility, and comparability of metabolomic data, particularly when chemotype assignment depends on metabolite abundance rather than qualitative composition alone.

In plant metabolomics, standardization should include quality control, randomization, control samples, description of preprocessing procedures, normalization, transformation, scaling, correction of batch effects, and reproducibility criteria [93,107,112]. NMR provides high structural and quantitative reproducibility in certain metabolomic contexts, although it generally has lower sensitivity than mass spectrometry for low-abundance compounds [113,114]. Therefore, the definition of chemotypes should explicitly state which fraction of metabolism was accessed by the technique employed, whether it comprised specific chemical classes, plant organs or structures, or global metabolomic fingerprints.

3.4.5. Quantification Versus Annotation

The proposal of chemotypes should not be based exclusively on tentatively annotated compounds. For a metabolite to be used as a chemotypic marker, multiple lines of evidence should be combined, including mass spectra, retention indices or retention time, MS/MS fragmentation, comparison with an authentic standard whenever possible, NMR in structurally critical cases, and validated quantification. The MSI standards propose confidence levels for metabolite identification, distinguishing confirmed identification, prob-

able annotation, putative chemical class, and unknown signals [107]. This logic has been refined by studies addressing the communication of uncertainty in high-resolution mass-spectrometry-based identification, reinforcing that different confidence levels should not be treated as equivalent [115–117].

The distinction between annotation and quantification has direct implications for the chemotype concept. A compound annotated with a low level of confidence may guide hypotheses, but should not, by itself, support a chemotypic category. In addition, relative abundance derived from chromatographic peak area is not necessarily equivalent to absolute concentration, especially when detector response, ionization efficiency, volatility, matrix effects, or extraction efficiency differ among compounds. For pharmacognostic, regulatory, or technological purposes, marker quantification should be supported by analytical procedures validated for selectivity, linearity, precision, accuracy, limit of detection, limit of quantification, and robustness [118,119]. Thus, chemotypes defined by chemical markers require detection, reliable identification, and, when applicable, validated quantification.

3.4.6. Statistical and Chemometric Support

Statistical analyses should be selected according to the structure of the chemical data and the design of the study. Exploratory methods, such as principal component analysis and hierarchical cluster analysis, may reveal preliminary patterns, but chemotype assignment should not rely solely on visual separation. Cluster solutions should be evaluated using alternative distance measures and clustering algorithms and should be supported by internal validation, resampling or bootstrap stability, and, whenever possible, external validation. PERMANOVA, mixed-effects models, or related approaches may be used to test predefined sources of variation, whereas supervised methods such as PLS-DA require cross-validation, permutation testing, transparent reporting of predictive performance, and independent validation whenever feasible [120–126].

When chemical data are expressed as relative abundances or percentages, their compositional structure should be explicitly considered. Appropriate transformations and statistical procedures are required to avoid spurious correlations caused by the constant-sum constraint [73]. Chemodiversity indices may complement chemotype analysis by describing chemical richness, evenness, structural dissimilarity, or biosynthetic relationships among metabolites, but they should not independently establish the existence of a chemotype [9,62,127].

The minimum methodological information required for transparent and reproducible chemotype assignment is summarized in Table 2.

Table 2. Minimum reporting checklist for plant chemotype studies.

Reporting Item	Minimum Information to Report	Purpose for Chemotype Attribution
Botanical identification/Voucher specimen	Updated scientific name, author citation, family, identification method and name of the specialist or reference used for taxonomic confirmation. Herbarium acronym, voucher number, collector, collection date and repository.	Ensures that chemical variation is interpreted within a correctly delimited taxon. Provides traceability, taxonomic verifiability and future reproducibility.
Plant organ	Organ or tissue used for chemotype determination (e.g., leaf, flower, inflorescence, stem, bark, root, seed, resin, glandular trichomes, or whole plant). Chemotype assignment should always be based on the same organ or tissue across all samples.	Ensures that organ-specific chemotypes are correctly identified and prevents organ-specific chemical variation from being misinterpreted as differences among chemotypes.

Table 2. Cont.

Reporting Item	Minimum Information to Report	Purpose for Chemotype Attribution
Sample size	Number of individuals, populations, accessions, replicates and independent collection events.	Supports recurrence, representativeness and statistical robustness.
Location	Country, region, locality, coordinates, altitude, habitat, cultivation or wild status and relevant environmental metadata.	Allows distinction between chemotype and chemical geotype or environmentally induced chemical phenotype.
Season and phenological stage	Season, month, climatic period, vegetative or reproductive stage and plant developmental stage.	Controls seasonal, ontogenetic and phenological variation.
Time of collection	Hour or time interval of sampling and, when relevant, light/dark period or circadian condition.	Avoids confusing circadian or daily metabolic variation with chemotypic differentiation.
Extraction method	Type of extraction, sample preparation, drying conditions, solvent or hydrodistillation method, extraction time, temperature and yield.	Controls methodological sources of chemical variation.
Analytical method	Analytical platform used, such as GC-MS, GC-FID, LC-MS/MS, UHPLC-HRMS, NMR or metabolomics, including column, detector, ionization mode and acquisition parameters.	Defines which fraction of the metabolome was accessed and enables analytical reproducibility.
Compound identification	Identification criteria, mass spectra, retention indices or retention times, MS/MS data, comparison with libraries, authentic standards and confidence level.	Prevents tentative annotations from being treated as definitive chemotypic markers.
Quantification	Relative or absolute quantification, calibration strategy, standards, response factors, validation parameters and units.	Distinguishes detection from reliable measurement of diagnostic markers.
Statistical analysis	Data preprocessing, normalization, transformation, scaling, treatment of compositional data, exploratory analyses, multivariate models, comparison of alternative clustering algorithms, linkage criteria and distance metrics, cluster-validity indices, resampling or bootstrap stability, permutation tests, and explicit criteria for group assignment.	Demonstrates whether chemical groups are statistically supported and not artifacts of sampling or modeling.
Supplementary data	Raw or processed chemical matrix, chromatograms, spectra, metadata table, statistical scripts, loading plots, marker lists and quality-control information.	Enables transparency, reanalysis, reproducibility and comparison among studies.
Cross-platform comparability	When multiple analytical platforms are used, report shared reference materials, quality-control strategy, feature alignment, normalization, and concordance across platforms.	Prevents platform-specific groupings from being presented as universal chemotypes and supports reproducible data integration.

3.5. Case Studies in Plant Chemotypes

Case studies demonstrate that the concept of chemotype is most robust when applied to recurrent, analytically reproducible, and biologically interpretable intraspecific chemical patterns. These cases also show that the notion of chemotype is not restricted to essential oils, although this is the field in which the term has been most widely used. Plant chemical diversity should be understood as a component of functional biodiversity, because individuals, populations, or lineages of the same species may produce distinct types or proportions of specialized metabolites in response to interactions among genotype, environment, space, time, and ecological organization [62,70]. This perspective makes it possible to distinguish chemotypes, ecotypes, chemical geotypes, chemical chronotypes, and chemical phenotypes as related, but not equivalent, expressions of plant chemodiversity. Representative case studies of plant chemotypes, organized by species, family, metabolic

class, chemical marker, evidence of stability, and main methodological limitation, are summarized in Supplementary Table S2.

3.5.1. Essential Oil Chemotypes

Essential oils represent the main historical and applied field in which the term chemotype has been used. Because they are complex mixtures, predominantly composed of mono- and sesquiterpenes, phenylpropanoids, and other specialized metabolites detectable in essential oils, they often exhibit intraspecific variation associated with the dominance of thymol, carvacrol, citral, geraniol, linalool, 1,8-cineole, methyl cinnamate, oxygenated sesquiterpenes, or terpene hydrocarbons. For example, essential oil chemotypes may distinguish plant materials with different aromatic, pharmacological, ecological, and industrial properties. However, the definition of essential oil chemotypes should not depend solely on the major compound, because essential oil composition may vary according to the environmental, developmental, phenological, organ-specific, seasonal, post-harvest, and analytical factors discussed above [17,76,87,128].

In *T. vulgaris*, one of the classic models of essential oil chemotypes, distinct chemical profiles have been recognized by the predominance of compounds such as thymol, carvacrol, linalool, geraniol, and other monoterpenes. Recent studies reinforce that these chemotypes do not merely represent aromatic variation but may also be associated with differences in bioactivity and biological mechanisms. Horváth et al. [129], for example, demonstrated that three essential oil chemotypes of *T. vulgaris* and their major compounds differentially modulate IL-6 and TNF α secretion in BV-2 microglia by interfering with pathways related to NF- κ B and C/EBP β . In a complementary way, Thompson et al. [63] discussed the chemical composition and biological activities of *Thymus vulgaris* essential oil, reinforcing the pharmacological and microbiological relevance of chemotypic variation in this species.

In *Lippia alba* (Mill.) N.E.Br. ex Britton & P.Wilson (Verbenaceae), essential oil chemotypes constitute another relevant example, especially because of the occurrence of profiles rich in citral, linalool, carvone, geraniol, and other characteristic chemical markers. The use of comprehensive two-dimensional gas chromatography (GC $\times$ GC) coupled with chemometrics enabled the discrimination of different *L. alba* chemotypes with higher analytical resolution, demonstrating that two-dimensional chromatographic techniques improve the characterization of complex essential oil profiles [130]. In addition, the identification of a new geraniol-rich chemotype in the Brazilian Amazon showed that seasonal variation can modify both the chemical composition and the antioxidant activity of the essential oil, indicating that chemotype recognition should consider temporal stability and ecological context [131]. Together, these studies show that chemotypes in aromatic plants may simultaneously have taxonomic, agronomic, pharmacognostic, and technological relevance.

Studies on *Piper* add a chemophenetic dimension to this debate by demonstrating that essential oil composition may vary according to season, circadian rhythm, plant organ, and cultivation condition. In *P. gaudichaudianum*, chemophenetic analysis combined with a reduction–oxidation index enabled the exploration of seasonal and circadian variation in leaf essential oil, showing that chemical composition should be interpreted at precise temporal scales and leading to the putative definition of nine chemotypes [87]. In a subsequent study, the comparison between wild specimens and monoclonal cultivars maintained for successive generations in two *Piper* species demonstrated that part of the chemical variation may result from chemical phenotypic plasticity, rather than necessarily from stable chemotypes [76]. This point is central for distinguishing chemotype from chemical chronotype, chemical geotype, and environmentally induced chemical phenotype.

Other aromatic species confirm the breadth of the problem. Zinno et al. [132] compared essential oils from two *Origanum vulgare* L. genotypes assigned to the carvacrol and thymol chemotypes and reported differences in their chemical composition and biological activities. The evaluation of samples cultivated in different European countries demonstrated that yield, composition, and chemotype distribution in *Origanum vulgare* may vary according to cultivation conditions and geographical origin, reinforcing the need to distinguish chemotype from chemical geotype [85]. In *Ocimum gratissimum* L. (Lamiaceae), chemical and olfactory analyses of concrete obtained in Madagascar indicate that sensory and chemical characterization can provide complementary information for evaluating aromatic materials [133]. In *Salvia rosmarinus* Spenn. (Lamiaceae), seasonal variability in essential oil composition and bioactivity reinforces that the classification of aromatic plant materials should be supported by adequate temporal sampling [134].

Additional well-characterized essential oil chemotypes have also been reported in species such as *Mentha longifolia* (L.) L. [135], *Artemisia herba-alba* Asso [136], *Camphora officinarum* Boerh. ex Fabr. (syn. *Cinnamomum camphora* (L.) J. Presl) [137], and several *Eucalyptus* species [138], further illustrating the broad taxonomic distribution and ecological relevance of chemotypic variation in aromatic plants.

3.5.2. Cannabinoid Chemotypes

Cannabis sativa represents a classic case of chemotypes based on non-volatile metabolites, particularly cannabinoids. The most widely used classification distinguishes THC-dominant, CBD-dominant, and intermediate materials according to the relative proportions of THC and CBD. However, although useful, this classification is insufficient to capture the chemical complexity of the species, which includes minor cannabinoids, terpenes, phenolics, nitrogen-containing compounds, and organ-specific metabolites [72,139]. Thus, the shift from a classification centered on THC and CBD toward a multivariate metabolomic approach has broadened the understanding of *Cannabis* chemotypes and chemovars.

Jin et al. [72] identified chemotypic markers in three *Cannabis* categories based on the specialized-metabolite profiles of inflorescences, leaves, stem bark, and roots, demonstrating that chemical classification depends on the organ analyzed and on the integration of different metabolic classes. In a complementary study, Jin et al. [72] evaluated phenotypic traits associated with the three chemotypic categories in the genus *Cannabis*, reinforcing that chemical composition may be linked to morphological and agronomic traits. These findings support the need to distinguish chemotype, chemical phenotype, and chemovar, especially in contexts of cultivation, medicinal production, and pharmaceutical standardization.

Recent multivariate approaches indicate that *Cannabis* classification should combine cannabinoid and terpene profiles. Birenboim et al. [140] demonstrated that multivariate chemovar classification can be improved by the joint use of terpene and cannabinoid profiles. Carvalho et al. [141], by evaluating varieties cultivated for medical purposes in southeastern Brazil, highlighted the importance of chemical profiling for traceability, quality control, and differentiation of plant materials. Herwig et al. [142] further showed that broad chemical fingerprints can discriminate strains and chemovars in the German market more accurately than traditional commercial categories. Together, these studies indicate that, in *Cannabis*, chemotype should be understood as a chemical category based on multiple markers, whereas chemovar tends to function as an agronomic, commercial, or regulatory category.

In addition to the classical THC-rich and CBD-rich chemotypes, less frequent categories are also relevant. Salamone et al. [143] phytochemically characterized chemo-

type V of *C. sativa*, identifying new dihydrophenanthrenoids capable of modulating the biosynthesis of lipid mediators in macrophages. This example demonstrates that *Cannabis* chemotypic diversity should not be reduced to the major cannabinoids. The integration of cannabinoids, terpenes, and other metabolites may have implications for bioactivity, safety, therapeutic efficacy, and the standardization of medicinal products. In this sense, proposals such as the Vigil Index of *Cannabis* Chemovars seek to relate systematic combinations of cannabinoids and terpenes to patient-reported outcomes, although such approaches should be interpreted cautiously with respect to clinical inference and variable control [144].

3.5.3. Alkaloid, Phenolic, and Glucosinolate Chemotypes

Non-volatile specialized metabolites also provide robust models for chemotype classification. Alkaloids constitute a structurally diverse class of specialized metabolites, with multiple biosynthetic origins and considerable pharmacological and ecological importance [145]. In *Mitragyna speciosa* Korth. (Rubiaceae), comparative metabolomic analyses revealed distinct alkaloid repertoires in young and mature leaves, demonstrating that tissue age and development can strongly modify chemical expression [146]. In *Piper fimbriatum* C. DC. (Piperaceae), computational metabolomics revealed previously overlooked chemodiversity of alkaloid scaffolds, indicating that classical methods may underestimate the chemical diversity of certain plant lineages [147].

Phenolic compounds also support relevant chemical classifications. Meng et al. [148] analyzed leaves from a core collection of *Eucommia ulmoides* Oliv. (Eucommiaceae) and used metabolomics to identify chemotypes and their diagnostic markers, demonstrating that phenolic profiles can discriminate chemical groups useful for conservation, germplasm selection, and breeding. These findings illustrate that non-volatile metabolites, like terpenes, can define biologically meaningful intraspecific chemical patterns.

Glucosinolates constitute one of the best-characterized examples of specialized metabolites giving rise to recurrent plant chemotypes, particularly within the Brassicaceae. In *Brassica napus* L., Missinou et al. [149] characterized variation in glucosinolate and phenolic composition across a broad genetic panel, revealing valuable chemical resources for breeding and chemical ecology. Gohain et al. [150] developed Vis-NIRS equations for the rapid quantification of glucosinolate content and composition in seeds from different chemotypes of Brassica oilseed crops. Katz et al. [151] demonstrated that genetic variation, environment, and demography interact to shape glucosinolate profiles in *Arabidopsis thaliana* L. across Europe, whereas Unger et al. [152] showed that glucosinolate structural diversity influences the recruitment of foliar bacterial communities. In *Arabidopsis halleri*, field sampling across 16 populations revealed two distinct glucosinolate chemotypes, with composition structured mainly by population affiliation and secondarily by geographic distance [153]. Additional well-characterized glucosinolate chemotypes occur in *Barbarea vulgaris* [154], where the polymorphism is heritable, and in *Bunias orientalis* [155], where contrasting glucosinolate profiles influence interactions with fungal pathogens. More recently, Le Boulch et al. [156] reported broad metabolomic diversity in collections of *Brassica rapa* L. and *Brassica oleracea* L. Collectively, these examples show that glucosinolate chemotypes should be delimited using reproducible qualitative or quantitative markers, with biological and genetic evidence used to strengthen, rather than obligatorily define, the assignment.

3.5.4. Chemotypes in Medicinal, Aromatic, and Food Plants

Across medicinal, aromatic, and food plants, chemotype information is most relevant when recurrent chemical differences affect biological activity, safety, organoleptic prop-

erties, or raw-material standardization. Representative applications are summarized in Supplementary Table S2; the corresponding methodological requirements are provided in Section 3.4 and Table 2.

3.6. Proposed Minimum Criteria and Levels of Evidence for Assigning Plant Chemotypes

The recognition of plant chemotypes ultimately depends on integrating chemical, biological, and methodological evidence. Rather than relying on isolated chemical markers, robust chemotype assignment requires reproducible chemical patterns interpreted in the context of taxonomy, sampling design, environmental variation, analytical standardization, and, whenever possible, ecological, biosynthetic, or genetic support. This integrative perspective reinforces the role of chemotypes as biologically meaningful categories for interpreting intraspecific chemodiversity rather than merely operational chemical classifications.

3.6.1. Proposed Minimum Criteria

The first criterion is botanical. No chemotype should be proposed without verifiable taxonomic identification, updated scientific nomenclature, a voucher deposited in a recognized herbarium, and traceability of the origin of the plant material. In research involving medicinal plants and natural products, incorrect species identification compromises the validity of any chemical, pharmacological, ecological, or regulatory inference [97,100]. Voucher specimens are indispensable because they allow taxonomic reassessment, confirmation of botanical identity, tracing of the analyzed material, and study reproducibility [98,99]. In addition, good agricultural and collection practice guidelines for medicinal plants reinforce the need to control the identity, provenance, collection, processing, and storage of plant raw material [103]. Therefore, without botanical authentication, a supposed chemotypic difference may merely reflect a taxonomic error, species mixture, unresolved synonymy, hybridization, or interspecific variation.

The second criterion is chemical and should be adjusted to the metabolic class under investigation. In essential oils, terpenoids, and volatile phenylpropanoids, chemotypic classification should consider chromatographic profile, mass spectra, retention indices, relative or absolute quantification, sampling replication, and control of factors that influence volatile composition. Constituent identification by GC-MS should be supported by specialized libraries, comparison of retention indices, and, whenever possible, authentic standards [109,110]. ISO 11024-1:1998 and ISO 9235:2021 reinforce the importance of chromatographic profiles and standardized terminology for natural aromatic raw materials [55,59]. However, because essential oil composition is strongly affected by the environment, phenology, plant organ, seasonality, and processing, the isolated major compound should not be considered sufficient to define a chemotype [17,76,87]. Studies on *T. vulgare* demonstrate that metabolomic fingerprints can preserve the distinction among chemotypes while also capturing broader chemical variation than that explained by dominant compounds alone [21].

In *Cannabis sativa*, the chemical criterion should integrate major cannabinoids, minor cannabinoids, terpenoids, phenolics, and other relevant metabolites, avoiding a classification restricted to THC-dominant, CBD-dominant, and intermediate categories. The inheritance of the chemical phenotype in *Cannabis* has been experimentally demonstrated, but chemotypic expression depends on the interaction among genetics, plant organ, cultivation conditions, and the metabolic class analyzed [66]. Metabolomic approaches indicate that the classification of *Cannabis* materials can be improved by integrating cannabinoids and terpenes, as well as markers present in inflorescences, leaves, stem bark, and roots [67,71,72,139,140,157]. Thus, the cannabinoid chemotype should be under-

stood as a category based on multiple chemical markers, and not merely on a simplified THC/CBD ratio.

For alkaloids, phenolics, and glucosinolates, chemical criteria should incorporate structural diversity, biosynthetic routes, organs analyzed, tissue age, annotation levels, and quantification. Alkaloids display great diversity in their scaffolds and structure-forming biosynthetic steps, making classification based only on the presence or absence of a single compound inadequate [145]. Studies on *Mitragyna speciosa* Korth. show that young and mature leaves may differ in their alkaloid repertoires, indicating a strong ontogenetic influence on the chemical profile [146]. In *Piper fimbriatum* C.DC., computational metabolomics revealed overlooked chemodiversity of alkaloid scaffolds, reinforcing that broader analytical methods can modify the perception of chemical groups [147]. For phenolics, studies on *Eucommia ulmoides* Oliv. and *Brassica napus* L. demonstrate that metabolomic approaches can identify chemotypes and markers associated with germplasm collections, chemical ecology, and plant breeding [148,149]. In the case of glucosinolates, structural variation is directly related to chemical defense, interaction ecology, and the selection of genetic resources, and is modulated by genetics, environment, and demography [149–152].

The third criterion involves reproducibility, biological coherence, and methodological support. A chemotype should be recurrent across independent samples, distinct individuals, populations, or comparable collection events. In addition, it should have biosynthetic or functional coherence; that is, the discriminant metabolites should be interpretable in relation to metabolic pathways, chemical families, enzymatic modules, ecological mechanisms, or plausible genetic patterns. In *T. vulgaris*, the genetic control of essential oil polymorphism provides a classical model of strongly supported chemotypes [18], whereas subsequent studies demonstrated that chemotypes involve qualitative and quantitative patterns of monoterpene co-occurrence [63]. Statistical interpretation should also be appropriate to the nature of the data, especially when relative proportions or chromatographic peak areas are used, because compositional data require specific treatment to avoid spurious correlations and analytical distortions [73]. In metabolomics, minimum standards for analysis and statistical validation should include transparent preprocessing, quality control, validation of supervised models, and caution with PLS-DA, especially because of the risk of overfitting [121,124,126].

The fourth criterion is integration with chemodiversity metrics and ecological interpretation. Chemotypic classification should not merely separate groups but should explain the meaning of the chemical difference. The incorporation of chemodiversity indices makes it possible to assess metabolite richness, relative abundance, structural dissimilarity, and biosynthetic relationships among compounds [9,127]. This approach expands the interpretation of chemotypes beyond the dominant marker by connecting chemical composition, ecological function, phenotypic plasticity, and chemical niche dynamics [7]. Recent studies on *T. vulgare* reinforce this need by demonstrating that chemotype can interact with maternal genotype, field neighborhood, plant performance, and resource allocation, without exhausting the complexity of the metabolic fingerprint [21,27].

3.6.2. Levels of Evidence for Chemotype Assignment

Based on the previous four criteria, we propose that the assignment of plant chemotypes should be organized into three levels of evidence: putative chemotype, supported chemotype, and confirmed chemotype (Figure 2). This gradation explicitly communicates the degree of confidence associated with the classification, preventing preliminary observations from being presented as consolidated biological categories. The adoption of levels of evidence is important because chemical identification may involve variable

degrees of certainty, ranging from unknown signals and tentative annotations to compounds confirmed by authentic standards or complementary structural techniques. The Metabolomics Standards Initiative proposed minimum requirements for chemical analysis and metabolite identification, whereas a widely used language for communicating confidence levels in high-resolution mass-spectrometry-based identification has been established [107,115,116].

Level 1 corresponds to the putative chemotype. In this category, there is initial evidence of a distinct chemical profile, but with relevant limitations, such as low replication, absence of population sampling, tentative identification of markers, lack of environmental control, absence of common-garden experiments, insufficient robust statistical validation, or no confirmation by authentic standards (Figure 1). This level is appropriate for exploratory studies, preliminary surveys, metabolomic screenings, and hypotheses of chemical differentiation. However, a putative chemotype should not be treated as a consolidated biological category. Uncertainty should be made explicit, especially when markers are grouped only by presence and absence, annotated by mass spectrometry without structural confirmation, or not supported by validated quantification [107,115,116].

Level 2 corresponds to the supported chemotype. In this category, the chemical pattern is recurrent across independent samples, presents clear chemical markers, has a described sampling design, receives statistical or chemometric support, and demonstrates biological coherence. Studies based on metabolomic fingerprints, multiple plant organs, multivariate validation, and discriminant markers fall within this level when there is still no direct demonstration of inheritance, stability under common conditions, or complete analytical validation of the markers (Figure 1). Methodological examples include the identification of chemotypic markers in three *Cannabis* categories based on metabolites from different plant organs, and the analysis of metabolomic fingerprints in *T. vulgare*, in which chemotype was related to parental genotype and broader metabolic variation [21,72]. The incorporation of compositional analysis and chemodiversity indices strengthens this level, as it allows evaluation not only of group separation, but also of richness, abundance, and structural relationships among metabolites [73,127].

Level 3 corresponds to the confirmed chemotype. In this category, classification should combine a reproducible chemical profile, relative stability under common or comparable conditions, a chemical marker identified and quantified through a validated method, demonstrated genetic or biosynthetic support, and functional or ecological coherence. Classic studies on *T. vulgaris* and *C. sativa* exemplify this level when they demonstrate, respectively, genetic control of essential oil polymorphism and inheritance of the cannabinoid chemical phenotype [18,66]. For pharmacognostic, industrial, or regulatory applications, confirmation should consider analytical-validation guidelines, including selectivity, linearity, precision, accuracy, robustness, limit of detection, and limit of quantification, as recommended by ICH Q2(R2) and ICH Q14 [119]. In a strict sense, a confirmed chemotype is not merely a statistically separated chemical group, but an intraspecific chemical entity supported by integrated evidence of botanical identity, stability, reproducibility, validated markers, and biological basis.

The structure of minimum criteria and levels of evidence preserves the usefulness of the chemotype concept without turning it into a generic descriptor for any chemical difference, as is recurrently observed in the literature. When the evidence is preliminary, the category should be presented as putative; when there is recurrence, clear markers, and statistical support, it should be presented as supported; and when there is stability, analytical validation, and a genetic or biosynthetic basis, it should be presented as confirmed.

The gradation makes scientific language more precise, facilitates comparison among studies, improves the reproducibility of phytochemical research, and strengthens applica-

tions in pharmacognosy, chemical ecology, conservation, agriculture, domestication, quality control, and natural-product development. In addition, it should always be specified that the chemotype is associated with the type of extract, the plant organ or part analyzed, and the analytical methods or platforms employed.

Chemotype naming should clearly define the name based on the chemical marker or markers that characterize it, avoiding generic nomenclatures frequently used in the literature, such as “Chemotype A” and/or “Chemotype B”. Instead, descriptive names are recommended, such as “compound A chemotype” and/or “compound A/B chemotype”.

3.7. Limitations and Challenges Across Analytical Platforms and Plant Taxa

No universal platform or numerical threshold can define chemotypes across all plant systems. GC-based methods favor volatile and semi-volatile constituents; LC-MS expands non-volatile coverage but is sensitive to ionization, matrix effects, and annotation uncertainty; and NMR provides robust structural and quantitative information at lower sensitivity. Consequently, groups inferred on different platforms may not be directly equivalent without shared standards, quality controls, harmonized preprocessing, and cross-platform validation [93,107,112–116].

Taxa also differ in organ specialization, pathway complexity, ploidy, hybridization, developmental timing, clonal structure, and environmental responsiveness. A dominant-compound rule suitable for an essential oil may be inappropriate for cannabinoid, alkaloid, phenolic, or glucosinolate systems, in which ratios, structural classes, or multivariate fingerprints may be more informative. Common-garden experiments, large population samples, or genetic validation may also be impractical for rare, long-lived, or poorly domesticated taxa [9,21,66,146–152,154–156].

The framework should therefore be applied as an evidence-based guide rather than as a universal algorithm. Authors should state the taxonomic scope, organ, extraction, platform, marker-confidence level, validation strategy, and limits of external generalization. Figure 2 and the expanded checklist in Table 2 provide a transparent basis for communicating these constraints.

3.8. Implications for Plant Biology and Applied Sciences

A rigorous definition of plant chemotypes has direct implications for ecology and evolution, since intraspecific chemical variation can modulate multiple biological interactions and adaptation to heterogeneous environments. Specialized metabolites should not be interpreted merely as end products of biosynthetic pathways, but as functional mediators of ecological interactions and components of the plant chemical phenotype [5–7]. In this sense, chemotypes can be useful categories for investigating how different chemical strategies are maintained in natural populations, how they are shaped by biotic and abiotic selection, and how they contribute to the occupation of ecological niches [62].

Recent literature on chemodiversity reinforces that intraspecific chemical diversity can influence measurable ecological processes, and not merely describe phytochemical differences. Theoretical and empirical studies show that chemodiversity can affect floral visitors, germination, growth, reproduction, herbivory, resource allocation, and plant performance in experimental communities [9,27,158,159]. In *T. vulgare*, intraspecific chemical variation interacts with maternal genotype, field neighborhood, and resource allocation, indicating that chemotype can explain part of the ecological variation, but does not exhaust the complexity of the chemical phenotype [27]. From an evolutionary perspective, recent quantitative models indicate that chemodiversity may be maintained by diversifying selection, pleiotropy, biosynthetic constraints, ecological interactions, and population structure [160–162]. Thus, chemotypes should be treated as hypotheses about intraspe-

cific chemical organization, whose ecological validity depends on functional, genetic, and environmental evidence.

Chemotypic variation may also influence the ecology of invasive plants. In invasive species, intraspecific differences in specialized metabolites can alter interactions with herbivores, pathogens, competitors, and soil microorganisms, thereby affecting invasion success and ecological impacts. Studies on *Bunias orientalis*, for example, demonstrate that glucosinolate chemotypes are associated with differences in plant–enemy interactions, highlighting the importance of chemotypic diversity for invasion ecology and community assembly [155].

In conservation biology, chemotype broadens the scope of diversity that should be preserved. Conserving medicinal, aromatic, and food plants cannot be limited to species occurrence because populations may harbor distinct chemical profiles, ecological functions, pharmacological potential, and cultural value. The loss of a chemotype may therefore represent the loss of an adaptive resource, ecological function, raw material, or associated knowledge [70,163].

The conservation of chemodiversity therefore requires strategies that incorporate population sampling, georeferencing, environmental metadata, chemical profiles, genetic diversity, and ecological knowledge. Studies of chemical biogeography demonstrate that widely distributed species may exhibit relevant spatial and temporal variation, making conservation based on only a few populations or on chemically uncharacterized samples inadequate [64]. In agricultural and food species, broad panels of chemical diversity, such as those described for phenolics and glucosinolates in *Brassica napus*, may reveal valuable genetic resources for chemical ecology, breeding, and food security [149]. In addition, chemodiversity indices can help identify priority populations for conservation by considering metabolite richness, relative abundance, and structural dissimilarity among compounds [9,62,161].

In pharmacognosy, phytotherapy, and natural products, chemotypic precision is essential for quality, safety, and efficacy. Medicinal species may display distinct chemical profiles capable of altering pharmacological activity, potency, toxicological risk, stability, drug interactions, and technological performance. For this reason, quality-control methods should integrate botanical identification, good agricultural and collection practices, traceability, chromatographic and metabolomic analysis, analytical validation, and acceptance criteria for chemical markers [102,103,164–166]. In essential oils, chemotypic variation can modify antimicrobial, anti-inflammatory, antioxidant, and toxicological activities, requiring precise chemical characterization before any pharmacological inference or therapeutic formulation [167,168].

In agriculture and domestication, chemotypes are relevant for genotype selection, productive stability, raw-material quality, traceability, and the organization of production chains. In aromatic species, the selection of genotypes with seasonal stability in essential oil composition is essential to ensure predictable composition and industrial value. In *L. alba*, the selection of genotypes of the citral chemotype with seasonal stability demonstrates how chemical classification can guide breeding and cultivation programs [169]. Approaches using GC × GC and chemometrics have also enabled the discrimination of new *L. alba* genotypes, increasing the analytical resolution applied to agronomic selection [130]. In *Origanum vulgare*, differences in yield, composition, and chemotypes among materials cultivated in different European countries reinforce that the environment, management, and germplasm origin should be considered in agricultural standardization [85].

Domestication can profoundly alter the chemical architecture of plants, either through direct selection of metabolites of interest or through indirect effects on growth, defense, aroma, flavor, toxicity, or productivity. Plant metabolomics applied to breeding makes it

possible to associate chemical profiles with genotypes, agronomic phenotypes, and productive performance, creating opportunities for metabolite-marker-assisted selection and genomic prediction of specialized metabolites [170–172]. In *Cannabis*, chemometric analyses have related cannabinoid composition to domestication processes, whereas recent pangenome studies indicate that domesticated cannabinoid synthases coexist with broad mosaic genomic variation, reinforcing the need to distinguish genotype, chemotype, and chemovar in agricultural and medicinal systems [68,173].

3.9. Future Perspectives

Future perspectives for the study of plant chemotypes depend on the integration of chemophenetics, genomics, transcriptomics, proteomics, metabolomics, chemical ecology, and environmental modeling. The definition of chemotype needs to move beyond classifications based only on chromatograms or major compounds toward models capable of connecting the chemical phenotype, genotype, biosynthetic pathways, metabolic regulation, plant organ, environment, and temporal scale. This transition is consistent with chemophenetic and chemodiversity approaches, which propose interpreting specialized metabolites across multiple scales of space, time, and biological organization, from the individual to the community. In addition, more precise definitions are needed for multi-organ chemotypes, as well as for chemotypes associated with organs or plant parts other than leaves and aerial parts, which predominate in the literature [62].

Multi-omics integration offers a promising basis for identifying genes, enzymes, regulatory networks, and biosynthetic routes associated with chemotypes. Resources such as the Plant Metabolic Network and PMhub expand the possibility of connecting genomic and metabolomic data in plants, allowing the inference of metabolic pathways and functions across different lineages and species [174,175]. Computational methods already make it possible to predict plant metabolic pathways and genes related to specialized-metabolite biosynthesis, especially when they integrate genomics, transcriptomics, metabolomics, and functional annotation [176–178]. In addition, large-scale intra- and interspecific metabolic-profiling studies can reveal patterns of conservation, convergence, divergence, and chemical plasticity across different plant lineages [179].

Artificial intelligence and machine learning are likely to play an increasing role in the predictive modeling of chemotypes if they are applied to well-annotated datasets and independently validated. Although the literature is not yet specifically centered on “plant chemotype modeling”, advances in genotype–phenotype prediction, structural classification of natural products, computational metabolomics, and multi-omics integration provide a basis for this emerging field. Machine-learning models can be applied to the prediction of chemotypes from chemical, genetic, environmental, geographical, and phenological matrices if they are trained on comparable data and complete metadata [180–182]. Tools such as NPCClassifier demonstrate the potential of neural networks for the structural classification of natural products, whereas molecular-graph approaches may expand the representation of chemical similarity and biosynthetic relationships among metabolites [183,184].

Predictive modeling can also assist in identifying genes responsible for specialized metabolites, inferring bioactivity, classifying geographical origin, and predicting chemical stability under different cultivation conditions. The integration of artificial intelligence, multi-omics data, and medicinal-plant resources may accelerate the discovery of bioactive compounds, genotype selection, the domestication of desirable chemical profiles, and quality control of plant raw materials [177,178,185]. However, these applications depend on training with robust datasets, control of sampling bias, external validation, biological interpretation of models, and transparency regarding confidence levels in predictions. Without

these precautions, artificial intelligence may reproduce taxonomic, chemical, and methodological inconsistencies already present in the literature.

Standardization and open data sharing will be essential for future chemotype research. Beyond methodological harmonization, comparable studies require the deposition of curated chemical data and metadata in public repositories to enable reproducibility and large-scale comparative analyses.

Open repositories and platforms, such as GNPS, MetaboLights, Metabolomics Workbench, Plant Metabolic Network, and PMhub, allow the sharing of raw data, metadata, protocols, molecular networks, and annotations, promoting reproducibility and independent reanalysis [174,175,186–190]. Metadata-standardization tools, such as SMetaS, may also improve the organization and interoperability of metabolomic data [191]. For horticultural, medicinal, and aromatic plants, the integrated use of web resources in metabolomics may accelerate the comparison of chemical profiles, the identification of markers, and the construction of chemotype databases associated with provenance, cultivation, and use [192,193].

Future advances in chemotype research will depend on integrating standardized metabolomic workflows with open databases and computational approaches capable of linking chemical variation to ecological, evolutionary, and genomic processes. This integration will strengthen the role of chemotypes as reproducible biological descriptors of intraspecific chemodiversity.

4. Conclusions

In the silent intimacy of plants, chemistry does not appear as a simple accumulation of molecules, but as a living language of adaptation, evolutionary memory, and relationships among organisms, territory, and time. Each specialized metabolite can be understood as a functional trace of ecological pressures, biosynthetic trajectories, genetic inheritance, and environmental responses. However, when this language is translated into science, it requires more than contemplation: it requires method, conceptual delimitation, reproducible evidence, and biologically grounded interpretation.

In response to the central question of this work, a plant chemotype is defined by the existence of a distinguishable, recurrent, relatively stable, methodologically reproducible, and biologically interpretable intraspecific chemical pattern. It is not merely the presence of a major compound or an occasional quantitative difference among samples, but a consistent chemical signature expressed by individuals, populations, or lineages belonging to the same taxon, capable of revealing chemical differentiation with biosynthetic, ecological, genetic, pharmacognostic, or functional significance.

Thus, the chemotype should be understood as a category situated at the interface between the chemical phenotype, metabolic diversity, and biological organization. Its definition requires the convergence of chemical criteria, such as presence, absence, predominance, metabolic proportions, diagnostic markers, or multivariate fingerprints; biological criteria, such as recurrence, relative stability, biosynthetic coherence, and possible genetic support; and methodological criteria, such as adequate sampling, botanical authentication, traceability, control of environmental variables, analytical standardization, reliable quantification, and robust statistical analysis.

The conceptual boundary of the chemotype must be preserved. Variation induced by plant organ, ontogenetic stage, seasonality, circadian rhythm, geographical origin, agronomic management, post-harvest processing, or analytical platform should not automatically be classified as a chemotype. In such cases, terms such as chemical phenotype, chemical ecotype, chemical geotype, chemical chronotype, or chemovar may be more appro-

appropriate if they more precisely express the causal, temporal, spatial, or applied scale of the observed variation.

The main contribution of this review is to argue that the chemotype concept should be retained but subjected to more restrictive criteria [15,16]. When used imprecisely, the term loses explanatory value and becomes a label for any circumstantial chemical variation. When used rigorously, it becomes an integrative category capable of connecting chemophenetics, metabolomics, chemical ecology, pharmacognosy, plant breeding, quality control, raw-material traceability, and natural-product standardization.

Thus, the answer to the title is direct: what defines a chemotype in plants is not chemical difference alone, but a demonstrable, recurrent, relatively stable, and analytically reproducible chemical difference within a recognized taxonomic unit and an explicitly defined organ, tissue, extraction, and analytical context. Biological, biosynthetic, ecological, functional, or genetic evidence strengthens the interpretation and may elevate the level of confidence, but it should not be treated as an obligatory prerequisite. Circumstantial variation should therefore not be called a chemotype; chemotype assignment requires convergence of botanical, chemical, sampling, and statistical evidence sufficient to demonstrate a consistent intraspecific chemical identity within the dynamic diversity of plants.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijpb17080066/s1>, Table S1, search strategy, descriptors, and PRISMA-style flow for plant chemotype records published from 2021 to 2026; Table S2, representative volatile and non-volatile plant chemotype case studies, including markers, analytical platforms, evidence of stability, and principal limitations.

Author Contributions: Conceptualization, Y.J.R.; methodology, M.C.d.J., M.M.G.-S. and Y.J.R.; formal analysis, D.A.R., L.O.-S.A., T.d.N.d.S. and Y.J.R.; investigation, D.A.R., L.O.-S.A., T.d.N.d.S., O.M.B., C.d.C.-O., E.d.L.d.J., P.B.d.A. and R.C.P.; resources, D.d.L.M. and Y.J.R.; data curation, D.A.R.; writing—original draft preparation, M.C.d.J., M.M.G.-S., D.A.R., L.O.-S.A., T.d.N.d.S., O.M.B., C.d.C.-O., E.d.L.d.J., P.B.d.A. and R.C.P.; writing—review and editing, D.A.R., D.d.L.M. and Y.J.R.; supervision, Y.J.R.; project administration, Y.J.R.; funding acquisition, Y.J.R. All authors have read and agreed to the published version of the manuscript.

Funding: The authors declare that financial support was received for the research publication of this article. This study was supported by the Federal University of Bahia through the Young Researcher Fellowship (PRPPG 010/2024) and by CNPq/MCTI/FINEP/FNDCT/Ação Transversal/CT-Agro—01/2024, grant number 2629/24.

Data Availability Statement: No new primary datasets were generated in this conceptual review. The literature search strategy and the structured case-study synthesis are provided in Supplementary Tables S1 and S2, respectively.

Acknowledgments: The authors acknowledge the institutional support provided by their respective research institutions. During manuscript preparation, ChatGPT 5.5 (OpenAI) was used for language refinement and spelling correction. All outputs were critically reviewed and edited by the authors, who assume full responsibility for the final content. The authors also acknowledge the traditional and enchanted guardians of Planet Earth, whose strength inspired the systematization of these data and whose presence reaffirms the harmony of nature as the foundation of all beings.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript: ABNT, Brazilian Association of Technical Standards; CBD, cannabidiol; CBG, cannabigerol; CT, chemotype; DOI, digital object identifier; DNA, deoxyribonucleic acid; EMA, European Medicines Agency; EO, essential oil; ET, ecotype; GACP, good agricultural and collection practices; GC, gas chromatography; GC-FID, gas chromatog-

raphy with flame ionization detection; GC-MS, gas chromatography–mass spectrometry; GNPS, Global Natural Products Social Molecular Networking; GT, geotype; HCA, hierarchical cluster analysis; HPLC, high-performance liquid chromatography; ICH, International Council for Harmonisation; ISO, International Organization for Standardization; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MeSH, Medical Subject Headings; MS/MS, tandem mass spectrometry; MSI, Metabolomics Standards Initiative; NIST, National Institute of Standards and Technology; NMR, nuclear magnetic resonance; PCA, principal component analysis; PERMANOVA, permutational multivariate analysis of variance; PLS-DA, partial least squares discriminant analysis; PMID, PubMed identifier; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SPME-GC-MS, solid-phase microextraction coupled to gas chromatography–mass spectrometry; SSR, simple sequence repeat; THC, Δ^9 -tetrahydrocannabinol; TLC-GC/MS, thin-layer chromatography coupled to gas chromatography–mass spectrometry; TS, topic search; UHPLC-HRMS, ultra-high-performance liquid chromatography–high-resolution mass spectrometry; UHPLC-MS, ultra-high-performance liquid chromatography–mass spectrometry; URL, uniform resource locator; WHO, World Health Organization.

References

- Langenheim, J.H. Higher plant terpenoids: A phyto-centric overview of their ecological roles. *J. Chem. Ecol.* **1994**, *20*, 1223–1280. [[CrossRef](#)] [[PubMed](#)]
- Pichersky, E.; Lewinsohn, E. Convergent evolution in plant specialized metabolism. *Annu. Rev. Plant Biol.* **2011**, *62*, 549–566. [[CrossRef](#)] [[PubMed](#)]
- Weng, J.K.; Philippe, R.N.; Noel, J.P. The rise of chemodiversity in plants. *Science* **2012**, *336*, 1667–1670. [[CrossRef](#)] [[PubMed](#)]
- Erb, M.; Kliebenstein, D.J. Plant secondary metabolites as defenses, regulators, and primary metabolites: The blurred functional trichotomy. *Plant Physiol.* **2020**, *184*, 39–52. [[CrossRef](#)] [[PubMed](#)]
- Kessler, A.; Kalske, A. Plant secondary metabolite diversity and species interactions. *Annu. Rev. Ecol. Evol. Syst.* **2018**, *49*, 115–138. [[CrossRef](#)]
- Wetzel, W.C.; Whitehead, S.R. The many dimensions of phytochemical diversity: Linking theory to practice. *Ecol. Lett.* **2020**, *23*, 16–32. [[CrossRef](#)] [[PubMed](#)]
- Müller, C.; Junker, R.R. Chemical phenotype as important and dynamic niche dimension of plants. *New Phytol.* **2022**, *234*, 1168–1174. [[CrossRef](#)] [[PubMed](#)]
- Moore, B.D.; Andrew, R.L.; Külheim, C.; Foley, W.J. Explaining intraspecific diversity in plant secondary metabolites in an ecological context. *New Phytol.* **2014**, *201*, 733–750. [[CrossRef](#)] [[PubMed](#)]
- Petrén, H.; Anaia, R.A.; Aragam, K.S.; Bräutigam, A.; Eckert, S.; Heinen, R.; Jakobs, R.; Ojeda-Prieto, L.; Popp, M.; Sasidharan, R.; et al. Understanding the chemodiversity of plants: Quantification, variation and ecological function. *Ecol. Monogr.* **2024**, *94*, e1635. [[CrossRef](#)]
- Gobbo-Neto, L.; Lopes, N.P. Plantas medicinais: Fatores de influência no conteúdo de metabólitos secundários. *Quim. Nova* **2007**, *30*, 374–381. [[CrossRef](#)]
- Desjardins, A.E. Natural product chemistry meets genetics: When is a genotype a chemotype? *J. Agric. Food Chem.* **2008**, *56*, 7587–7592. [[CrossRef](#)] [[PubMed](#)]
- Barton, K.E.; Koricheva, J. The ontogeny of plant defense and herbivory: Characterizing general patterns using meta-analysis. *Am. Nat.* **2010**, *175*, 481–491. [[CrossRef](#)] [[PubMed](#)]
- Keefover-Ring, K.; Thompson, J.D.; Linhart, Y.B. Beyond six scents: Defining a seventh *Thymus vulgaris* chemotype new to southern France by ethanol extraction. *Flavour Fragr. J.* **2009**, *24*, 117–122. [[CrossRef](#)]
- Santesson, R. Lavar: Some aspects on lichen taxonomy. *Sven. Naturvetensk.* **1968**, *21*, 176–184.
- Polatoğlu, K. Chemotypes: A fact that should not be ignored in natural product studies. *Nat. Prod. J.* **2013**, *3*, 10–14. [[CrossRef](#)]
- Müller, C.; Dussarrat, T.; Van Dam, N.M. What is a plant chemotype, anyway? *Trends Plant Sci.* **2026**, in press. [[CrossRef](#)] [[PubMed](#)]
- Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G.; Scheffer, J.J.C. Factors affecting secondary metabolite production in plants: Volatile components and essential oils. *Flavour Fragr. J.* **2008**, *23*, 213–226. [[CrossRef](#)]
- Vernet, P.; Gouyon, P.H.; Valdeyron, G. Genetic control of oil polymorphism in *Thymus vulgaris* L. *Genetica* **1986**, *69*, 227–231. [[CrossRef](#)]
- Clemensen, A.K.; Provenza, F.D.; Hendrickson, J.R.; Grusak, M.A. Ecological implications of plant secondary metabolites: Phytochemical diversity can enhance agricultural sustainability. *Front. Sustain. Food Syst.* **2020**, *4*, 547826. [[CrossRef](#)]

20. Harborne, J.B.; Turner, B.L. *Plant Chemosystematics*; Academic Press: London, UK, 1984.
21. Dussarrat, T.; Schweiger, R.; Ziaja, D.; Nguyen, T.T.N.; Krause, L.; Jakobs, R.; Eilers, E.J.; Müller, C. Influences of chemotype and parental genotype on metabolic fingerprints of tansy plants uncovered by predictive metabolomics. *Sci. Rep.* **2023**, *13*, 11645. [[CrossRef](#)] [[PubMed](#)]
22. Peters, K.; Worrich, A.; Weinhold, A.; Alka, O.; Balcke, G.; Birkemeyer, C.; Bruelheide, H.; Calf, O.W.; Dietz, S.; Dührkop, K.; et al. Current challenges in plant eco-metabolomics. *Int. J. Mol. Sci.* **2018**, *19*, 1385. [[CrossRef](#)] [[PubMed](#)]
23. Bradshaw, A.D. Evolutionary significance of phenotypic plasticity in plants. *Adv. Genet.* **1965**, *13*, 115–155. [[CrossRef](#)]
24. Sultan, S.E. Phenotypic plasticity for plant development, function and life history. *Trends Plant Sci.* **2000**, *5*, 537–542. [[CrossRef](#)] [[PubMed](#)]
25. Hillig, K.W.; Mahlberg, P.G. A chemotaxonomic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). *Am. J. Bot.* **2004**, *91*, 966–975. [[CrossRef](#)] [[PubMed](#)]
26. Nguyen, L.M.N.; Ebersbach, J.; Razafimandimby, D.; Rasplus, J.-Y.; Uthe, H.; Rafaliarison, R.R.; Döll, S.; Poeschl, Y.; Valenta, K.; van Dam, N.M.; et al. Sources of variation in plant chemical diversity: Lessons from Malagasy *Ficus*. *Am. J. Bot.* **2025**, *112*, e70102. [[CrossRef](#)] [[PubMed](#)]
27. Ziaja, D.; Sasidharan, R.; Jakobs, R.; Eilers, E.J.; Müller, C. Chemotype, maternal genotype, or field neighbors: What influences performance and resource allocation in a perennial plant species the most? *Oecologia* **2025**, *207*, 134. [[CrossRef](#)] [[PubMed](#)]
28. Culberson, W.L. The use of chemistry in the systematics of the lichens. *Taxon* **1969**, *18*, 152–166. [[CrossRef](#)]
29. Lapage, S.P.; Sneath, P.H.A.; Lessel, E.F.; Skerman, V.B.D.; Seeliger, H.P.R.; Clark, W.A. (Eds.) *International Code of Nomenclature of Bacteria: Bacteriological Code, 1990 Revision*; ASM Press: Washington, DC, USA, 1992.
30. Parker, C.T.; Tindall, B.J.; Garrity, G.M. International Code of Nomenclature of Prokaryotes. *Int. J. Syst. Evol. Microbiol.* **2019**, *69*, S1–S111. [[CrossRef](#)] [[PubMed](#)]
31. Culberson, W.L.; Culberson, C.F. Habitat selection by chemically differentiated races of lichens. *Science* **1967**, *158*, 1195–1197. [[CrossRef](#)] [[PubMed](#)]
32. Culberson, C.F. Improved conditions and new data for the identification of lichen products by a standardized thin-layer chromatographic method. *J. Chromatogr. A* **1972**, *72*, 113–125. [[CrossRef](#)] [[PubMed](#)]
33. Brodo, I.M. Interpreting chemical variation in lichens for systematic purposes. *Bryologist* **1986**, *89*, 132–138. [[CrossRef](#)]
34. Hegnauer, R. *Chemotaxonomie der Pflanzen: Eine Übersicht über die Verbreitung und die Systematische Bedeutung der Pflanzenstoffe*; Birkhäuser: Basel, Switzerland, 1962.
35. Alston, R.E.; Turner, B.L. *Biochemical Systematics*; Prentice-Hall: Englewood Cliffs, NJ, USA, 1963.
36. Fairbrothers, D.E.; Mabry, T.J.; Scogin, R.L.; Turner, B.L. The bases of angiosperm phylogeny: Chemotaxonomy. *Ann. Mo. Bot. Gard.* **1975**, *62*, 765–800. [[CrossRef](#)]
37. Smith, P.M. *The Chemotaxonomy of Plants*; Edward Arnold: London, UK, 1976.
38. Gottlieb, O.R. *Micromolecular Evolution, Systematics and Ecology: An Essay into a Novel Botanical Discipline*; Springer: Berlin/Heidelberg, Germany, 1982. [[CrossRef](#)]
39. Guenther, E. *The Essential Oils, Vol. 1: History-Origin in Plants-Production-Analysis*; D. Van Nostrand Company: New York, NY, USA, 1948.
40. Wolffenbüttel, A.N. *Base da Química dos Óleos Essenciais e Aromaterapia: Abordagem Técnica e Científica*; Roca: São Paulo, Brazil, 2016.
41. Franchomme, P. *La Science des Huiles Essentielles Médicinales: Propriétés Pharmacologiques, Mécanismes d'Action, Applications Thérapeutiques*; Guy Trédaniel: Paris, France, 2021.
42. Franchomme, P.; Jollois, R.; Pénoël, D. *L'aromathérapie Exactement: Encyclopédie de L'utilisation Thérapeutique des Huiles Essentielles*; Roger Jollois: Limoges, France, 2001.
43. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils: A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [[CrossRef](#)] [[PubMed](#)]
44. Bouayed, J.; Bohn, T. (Eds.) *Nutrition, Well-Being and Health*; InTech: Rijeka, Croatia, 2012.
45. Granger, R.; Passet, J. Types chimiques (chénotypes) de l'espèce *Thymus vulgaris* L. *C. R. Acad. Sci. Paris* **1971**, *273*, 2350–2353.
46. Granger, R.; Passet, J.C. *Thymus vulgaris* spontané de France: Races chimiques et chénotypes. *Phytochemistry* **1973**, *12*, 1683–1691. [[CrossRef](#)]
47. Thompson, J.D.; Tarayre, M. Exploring the genetic basis and proximate causes of variation in female fertility advantage in gynodioecious *Thymus vulgaris*. *Evolution* **2000**, *54*, 1510–1520. [[CrossRef](#)] [[PubMed](#)]
48. Small, E.; Beckstead, H.D. Common cannabinoid phenotypes in 350 stocks of *Cannabis*. *Lloydia* **1973**, *36*, 144–165. [[PubMed](#)]
49. Small, E.; Cronquist, A. A practical and natural taxonomy for *Cannabis*. *Taxon* **1976**, *25*, 405–435. [[CrossRef](#)]
50. Hazekamp, A.; Fisedick, J.T. *Cannabis*: From cultivar to chemovar. *Drug Test. Anal.* **2012**, *4*, 660–667. [[CrossRef](#)] [[PubMed](#)]
51. Linhart, Y.B.; Thompson, J.D. Terpene-based selective herbivory by *Helix aspersa* (Mollusca) on *Thymus vulgaris* (Labiatae). *Oecologia* **1995**, *102*, 126–132. [[CrossRef](#)] [[PubMed](#)]

52. Lawrence, B.M. Chemical components of Labiatae oils and their exploitation. In *Advances in Labiatae Science*; Harley, R.M., Reynolds, T., Eds.; Royal Botanic Gardens, Kew: Richmond, UK, 1992; pp. 399–436.
53. Simões, C.M.O.; Schenkel, E.P.; Mello, J.C.P.; Mentz, L.A.; Petrovick, P.R. (Eds.) *Farmacognosia: Do Produto Natural ao Medicamento*, 1st ed.; Artmed: Porto Alegre, Brazil, 2017; 486p.
54. European Pharmacopoeia Commission. *Essential oils: General Monograph 2098 and Chapter 5.30: Monographs on Essential Oils*; EDQM: Strasbourg, France, 2021.
55. ISO 9235:2021; Aromatic Natural Raw Materials: Vocabulary. International Organization for Standardization (ISO): Geneva, Switzerland, 2021.
56. Elzinga, S.; Fischedick, J.; Podkolinski, R.; Raber, J.C. Cannabinoids and terpenes as chemotaxonomic markers in *Cannabis*. *Nat. Prod. Chem. Res.* **2015**, *3*, 181. [[CrossRef](#)]
57. Erkelens, J.L.; Hazekamp, A. That which we call Indica, by any other name would smell as sweet. *Cannabinoids* **2014**, *9*, 9–15.
58. Lewis, M.A.; Russo, E.B.; Smith, K.M. Pharmacological foundations of *Cannabis* chemovars. *Planta Med.* **2018**, *84*, 225–233. [[CrossRef](#)] [[PubMed](#)]
59. ISO 11024-1:1998; Essential Oils: General Guidance on Chromatographic Profiles: Part 1: Preparation of Chromatographic Profiles for Presentation in Standards. International Organization for Standardization (ISO): Geneva, Switzerland, 1998.
60. European Medicines Agency. *Community Herbal Monograph on Thymus vulgaris L. and Thymus zygis L., Herba*; EMA/HMPC/342332/2013; European Medicines Agency: London, UK, 2013.
61. Zidorn, C. Plant chemophenetics: A new term for plant chemosystematics/plant chemotaxonomy in the macro-molecular era. *Phytochemistry* **2019**, *163*, 147–148. [[CrossRef](#)] [[PubMed](#)]
62. Ramos, Y.J.; Gouvêa-Silva, J.G.; Machado, D.B.; Felisberto, J.S.; Pereira, R.C.; Sadgrove, N.J.; Moreira, D.L. Chemophenetic and chemodiversity approaches: New insights on modern study of plant secondary metabolite diversity at different spatiotemporal and organizational scales. *Rev. Bras. Farmacogn.* **2023**, *33*, 49–72. [[CrossRef](#)]
63. Thompson, J.D.; Chalchat, J.C.; Michelin, B.; Linhart, Y.B.; Ehlers, B. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *J. Chem. Ecol.* **2003**, *29*, 859–880. [[CrossRef](#)] [[PubMed](#)]
64. Keefover-Ring, K. The chemical biogeography of a widespread aromatic plant species shows both spatial and temporal variation. *Ecol. Evol.* **2022**, *12*, e9265. [[CrossRef](#)] [[PubMed](#)]
65. Fournier, G.; Richez-Dumanois, C.; Duvezin, J.; Mathieu, J.P.; Paris, M. Identification of a new chemotype in *Cannabis sativa*: Cannabigerol-dominant plants, biogenetic and agronomic prospects. *Planta Med.* **1987**, *53*, 277–280. [[CrossRef](#)] [[PubMed](#)]
66. De Meijer, E.P.M.; Bagatta, M.; Carboni, A.; Crucitti, P.; Moliterni, V.M.C.; Ranalli, P.; Mandolino, G. The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics* **2003**, *163*, 335–346. [[CrossRef](#)] [[PubMed](#)]
67. Hazekamp, A.; Tejkalová, K.; Papadimitriou, S. *Cannabis*: From cultivar to chemovar II: A metabolomics approach to *Cannabis* classification. *Cannabis Cannabinoid Res.* **2016**, *1*, 202–215. [[CrossRef](#)]
68. Mudge, E.M.; Murch, S.J.; Brown, P.N. Chemometric analysis of cannabinoids: Chemotaxonomy and domestication syndrome. *Sci. Rep.* **2018**, *8*, 13090. [[CrossRef](#)] [[PubMed](#)]
69. Eilers, E.J.; Kleine, S.; Eckert, S.; Waldherr, S.; Müller, C. Flower production, headspace volatiles, pollen nutrients, and florivory in *Tanacetum vulgare* chemotypes. *Front. Plant Sci.* **2021**, *11*, 611877. [[CrossRef](#)] [[PubMed](#)]
70. De Almeida, P.B.; Felisberto, J.S.; Villarreal, C.F.; Pereira, R.C.; Moreira, D.L.; Ramos, Y.J. Why are there so few studies on plant diversity chemical types?—how ecotypes and chemotypes can unlock insights into environmental and community health. *Int. J. Mol. Biol. Open Access.* **2024**, *7*, 28–30. [[CrossRef](#)]
71. Fischedick, J.T. Identification of terpenoid chemotypes among high (-)-trans- Δ^9 -tetrahydrocannabinol-producing *Cannabis sativa* L. cultivars. *Cannabis Cannabinoid Res.* **2017**, *2*, 34–47. [[CrossRef](#)] [[PubMed](#)]
72. Jin, D.; Henry, P.; Shan, J.; Chen, J. Identification of chemotypic markers in three chemotype categories of *Cannabis* using secondary metabolites profiled in inflorescences, leaves, stem bark, and roots. *Front. Plant Sci.* **2021**, *12*, 699530. [[CrossRef](#)] [[PubMed](#)]
73. Brückner, A.; Heethoff, M. A chemo-ecologists' practical guide to compositional data analysis. *Chemoecology* **2017**, *27*, 33–46. [[CrossRef](#)]
74. Pavarini, D.P.; Pavarini, S.P.; Niehues, M.; Lopes, N.P. Exogenous influences on plant secondary metabolite levels. *Anim. Feed Sci. Technol.* **2012**, *176*, 5–16. [[CrossRef](#)]
75. Kleine, S.; Müller, C. Differences in shoot and root terpenoid profiles and plant responses to fertilisation in *Tanacetum vulgare*. *Phytochemistry* **2013**, *96*, 123–131. [[CrossRef](#)] [[PubMed](#)]
76. Ramos, Y.J.; Felisberto, J.S.; Gouvêa-Silva, J.G.; de Souza, U.C.; da Costa-Oliveira, C.; de Queiroz, G.A.; Guimarães, E.F.; Sadgrove, N.J.; de Lima Moreira, D. Phenoplasticity of essential oils from two species of Piper (Piperaceae): Comparing wild specimens and bi-generational monoclonal cultivars. *Plants* **2022**, *11*, 1771. [[CrossRef](#)] [[PubMed](#)]

77. Pichersky, E.; Gang, D.R. Genetics and biochemistry of secondary metabolites in plants: An evolutionary perspective. *Trends Plant Sci.* **2000**, *5*, 439–445. [[CrossRef](#)] [[PubMed](#)]
78. Dewick, P.M. *Medicinal Natural Products: A Biosynthetic Approach*, 3rd ed.; John Wiley & Sons: Chichester, UK, 2009.
79. Nützmann, H.W.; Osbourn, A. Gene clustering in plant specialized metabolism. *Curr. Opin. Biotechnol.* **2014**, *26*, 91–99. [[CrossRef](#)] [[PubMed](#)]
80. Turesson, G. The genotypical response of the plant species to the habitat. *Hereditas* **1922**, *3*, 211–350. [[CrossRef](#)]
81. Clausen, J.; Keck, D.D.; Hiesey, W.M. *Experimental Studies on the Nature of Species. I. Effect of Varied Environments on Western North American Plants*; Carnegie Institution of Washington: Washington, DC, USA, 1940.
82. Kawecki, T.J.; Ebert, D. Conceptual issues in local adaptation. *Ecol. Lett.* **2004**, *7*, 1225–1241. [[CrossRef](#)]
83. Lowry, D.B. Ecotypes and the controversy over stages in the formation of new species. *Biol. J. Linn. Soc.* **2012**, *106*, 241–257. [[CrossRef](#)]
84. Buckley, J.; Pashalidou, F.G.; Fischer, M.C.; Widmer, A.; Mescher, M.C.; De Moraes, C.M. Divergence in glucosinolate profiles between high- and low-elevation populations of *Arabidopsis halleri* correspond to variation in field herbivory and herbivore behavioral preferences. *Int. J. Mol. Sci.* **2019**, *20*, 174. [[CrossRef](#)] [[PubMed](#)]
85. Raal, A.; Gontova, T.; Ivask, A.; Orav, A.; Koshovyi, O. Yield, composition, and chemotypes of essential oils from *Origanum vulgare* L. aerial parts cultivated in different European countries. *Agronomy* **2024**, *14*, 3046. [[CrossRef](#)]
86. Ramos, Y.J.; Machado, D.B.; Queiroz, G.A.; Guimarães, E.F.; Defaveri, A.C.A.; Moreira, D.L. Chemical composition of the essential oils of circadian rhythm and of different vegetative parts from *Piper mollicomum* Kunth: A medicinal plant from Brazil. *Biochem. Syst. Ecol.* **2020**, *92*, 104116. [[CrossRef](#)]
87. Ramos, Y.J.; Costa-Oliveira, C.d.; Candido-Fonseca, I.; Queiroz, G.A.d.; Guimarães, E.F.; Defaveri, A.C.A.e.; Sadgrove, N.J.; Moreira, D.d.L. Advanced chemophenetic analysis of essential oil from leaves of *Piper gaudichaudianum* Kunth (Piperaceae) using a new reduction-oxidation index to explore seasonal and circadian rhythms. *Plants* **2021**, *10*, 2116. [[CrossRef](#)] [[PubMed](#)]
88. Câmara, M.B.P.; Lima, A.S.; Jumbo, L.O.V.; Tavares, C.P.; Mendonça, C.d.J.S.; Monteiro, O.S.; Araújo, S.H.C.; Oliveira, E.E.d.; Lima Neto, J.S.; Maia, J.G.S.; et al. Seasonal and circadian evaluation of the *Pectis brevipedunculata* essential oil and its acaricidal activity against *Rhipicephalus microplus* (Acari: Ixodidae). *J. Braz. Chem. Soc.* **2023**, *34*, 1020–1029. [[CrossRef](#)]
89. Do Rosário, C.J.R.M.; Lima, A.d.S.; Soares, I.S.; Araújo, R.d.Q.; Coimbra, V.C.S.; Andrade, E.H.d.A.; Sousa, D.M.; Figueiredo, P.L.B.; Costa-Junior, L.M.; da Rocha, C.Q. Seasonal and circadian evaluation of *Ageratum conyzoides* essential oil and its nematocidal activity against *Caenorhabditis elegans*. *Acta Trop.* **2024**, *256*, 107274. [[CrossRef](#)] [[PubMed](#)]
90. Kliebenstein, D.J.; Lambrix, V.M.; Reichelt, M.; Gershenzon, J.; Mitchell-Olds, T. Gene duplication in the diversification of secondary metabolism: Tandem 2-oxoglutarate-dependent dioxygenases control glucosinolate biosynthesis in *Arabidopsis*. *Plant Cell* **2001**, *13*, 681–693. [[CrossRef](#)] [[PubMed](#)]
91. de Medeiros, E.A.D.P.; Nascimento, R.V.; Duarte-Junior, E.B.; Pereira, C.d.S.B.; Moreira, Y.N.; Torre, R.; Velez, A.S.M.M.; Decote-Ricardo, D.; de Lima, M.E.F.; dos Santos, A.M.; et al. Chemodiversity and Trypanocidal Activity of the Volatile Oils of Tropical Black Sage. *Rev. Bras. Farmacogn.* **2024**, *34*, 1101–1115. [[CrossRef](#)]
92. Holopainen, M.; Hiltunen, R.; von Schantz, M. A study on tansy chemotypes. *Planta Med.* **1987**, *53*, 284–287. [[CrossRef](#)] [[PubMed](#)]
93. Fiehn, O.; Wohlgemuth, G.; Scholz, M.; Kind, T.; Lee, D.Y.; Lu, Y.; Moon, S.; Nikolau, B. Quality control for plant metabolomics: Reporting MSI-compliant studies. *Plant J.* **2008**, *53*, 691–704. [[CrossRef](#)] [[PubMed](#)]
94. Kim, H.K.; Verpoorte, R. Sample preparation for plant metabolomics. *Phytochem. Anal.* **2010**, *21*, 4–13. [[CrossRef](#)] [[PubMed](#)]
95. Rodrigues, A.M.; Ribeiro-Barros, A.I.; António, C. Experimental design and sample preparation in forest tree metabolomics. *Metabolites* **2019**, *9*, 285. [[CrossRef](#)] [[PubMed](#)]
96. Da Silva, W.B.; Hispagnol, G.F.; dos Santos Nunes, E.V.; Castro-Gamboa, I.; Pilon, A.C. Plant sample preparation for metabolomics, lipidomics, ionomics, fluxomics, and peptidomics. *Separations* **2025**, *12*, 21. [[CrossRef](#)]
97. Bennett, B.C.; Balick, M.J. Phytomedicine 101: Plant taxonomy for preclinical and clinical medicinal plant researchers. *J. Soc. Integr. Oncol.* **2008**, *6*, 150–157. [[CrossRef](#)]
98. Eisenman, S.W.; Tucker, A.O.; Struwe, L. Voucher specimens are essential for documenting source material used in medicinal plant investigations. *J. Med. Act. Plants* **2012**, *1*, 30–43.
99. Culley, T.M. Why vouchers matter in botanical research. *Appl. Plant Sci.* **2013**, *1*, 1300076. [[CrossRef](#)] [[PubMed](#)]
100. Rivera, D.; Allkin, R.; Obón, C.; Alcaraz, F.; Verpoorte, R.; Heinrich, M. What is in a name? The need for accurate scientific nomenclature for plants. *J. Ethnopharmacol.* **2014**, *152*, 393–402. [[CrossRef](#)] [[PubMed](#)]
101. Funk, V.A.; Gastel, M.R.; Devine, A.; Kellogg, C.L.; Wurdack, K.J.; Tuccinardi, C.; Radosavljevic, A.; Peters, M.; Coddington, J.A. Guidelines for collecting vouchers and tissues intended for genomic work. *Biodivers. Data J.* **2017**, *5*, e11625. [[CrossRef](#)] [[PubMed](#)]
102. World Health Organization. *Quality Control Methods for Medicinal Plant Materials*; WHO: Geneva, Switzerland, 1998.
103. World Health Organization. *WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants*; WHO: Geneva, Switzerland, 2003.

104. Soejarto, D.D.; Addo, E.M.; Kinghorn, A.D. The continuing need for taxonomic input in phytochemical research. *J. Ethnopharmacol.* **2026**, *354*, 120474. [[CrossRef](#)] [[PubMed](#)]
105. Sadgrove, N.J.; Padilla-González, G.F.; Phumthum, M. Fundamental chemistry of essential oils and volatile organic compounds, methods of analysis and authentication. *Plants* **2022**, *11*, 789. [[CrossRef](#)] [[PubMed](#)]
106. Morrison, N.; Bearden, D.; Bundy, J.G.; Collette, T.; Currie, F.; Davey, M.P.; Haigh, N.S.; Hancock, D.; Jones, O.A.H.; Rochfort, S.; et al. Standard reporting requirements for biological samples in metabolomics experiments: Environmental context. *Metabolomics* **2007**, *3*, 203–210. [[CrossRef](#)]
107. Sumner, L.W.; Amberg, A.; Barrett, D.; Beale, M.H.; Beger, R.; Daykin, C.A.; Fan, T.W.M.; Fiehn, O.; Goodacre, R.; Griffin, J.L.; et al. Proposed minimum reporting standards for chemical analysis: Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* **2007**, *3*, 211–221. [[CrossRef](#)] [[PubMed](#)]
108. Van den Dool, H.; Kratz, P.D. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr. A* **1963**, *11*, 463–471. [[CrossRef](#)] [[PubMed](#)]
109. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, USA, 2007.
110. Babushok, V.I.; Linstrom, P.J.; Zenkevich, I.G. Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* **2011**, *40*, 043101. [[CrossRef](#)]
111. Baser, K.H.C.; Buchbauer, G. (Eds.) *Handbook of Essential Oils: Science, Technology, and Applications*; CRC Press: Boca Raton, FL, USA, 2010.
112. Wolfender, J.L.; Marti, G.; Queiroz, E.F. Advances in techniques for profiling crude extracts and for the rapid identification of natural products: Dereplication, quality control and metabolomics. *Curr. Org. Chem.* **2010**, *14*, 1808–1832. [[CrossRef](#)]
113. Hall, R. Plant metabolomics: The missing link in functional genomics strategies. *Plant Cell* **2002**, *14*, 1437–1440. [[CrossRef](#)] [[PubMed](#)]
114. Ocampos, F.M.M.; de Souza, A.J.B.; Ribeiro, G.H.; Almeida, L.S.; Cónsolo, N.R.B.; Colnago, L.A. NMR-based plant metabolomics protocols: A step-by-step guide. *Front. Nat. Prod.* **2024**, *3*, 1414506. [[CrossRef](#)]
115. Schymanski, E.L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H.P.; Hollender, J. Identifying small molecules via high resolution mass spectrometry: Communicating confidence. *Environ. Sci. Technol.* **2014**, *48*, 2097–2098. [[CrossRef](#)] [[PubMed](#)]
116. Salem, M.A.; Perez de Souza, L.; Serag, A.; Fernie, A.R.; Farag, M.A.; Ezzat, S.M.; Alseekh, S. Metabolomics in the context of plant natural products research: From sample preparation to metabolite annotation. *Metabolites* **2020**, *10*, 37. [[CrossRef](#)] [[PubMed](#)]
117. Metz, T.O.; Chang, C.H.; Gautam, V.; Anjum, A.; Tian, S.; Wang, F.; Colby, S.M.; Nunez, J.R.; Blumer, M.R.; Edison, A.S.; et al. Introducing “identification probability” for automated and transferable assessment of metabolite identification confidence in metabolomics and related studies. *Anal. Chem.* **2025**, *97*, 1–11. [[CrossRef](#)] [[PubMed](#)]
118. Sadek, P.C. *The HPLC Solvent Guide*, 2nd ed.; Wiley-Interscience: New York, NY, USA, 2002.
119. International Council for Harmonisation. *ICH Guideline Q2(R2): Validation of Analytical Procedures*; International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Geneva, Switzerland, 2023.
120. Anderson, M.J. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **2001**, *26*, 32–46. [[CrossRef](#)]
121. Goodacre, R.; Broadhurst, D.; Smilde, A.K.; Kristal, B.S.; Baker, J.D.; Beger, R.; Bessant, C.; Connor, S.; Capuani, G.; Craig, A.; et al. Proposed minimum reporting standards for data analysis in metabolomics. *Metabolomics* **2007**, *3*, 231–241. [[CrossRef](#)]
122. Legendre, P.; Legendre, L. *Numerical Ecology*, 3rd ed.; Elsevier: Amsterdam, The Netherlands, 2012.
123. Borcard, D.; Gillet, F.; Legendre, P. *Numerical Ecology with R*, 2nd ed.; Springer: Cham, Switzerland, 2018.
124. Szymańska, E.; Saccenti, E.; Smilde, A.K.; Westerhuis, J.A. Double-check: Validation of diagnostic statistics for PLS-DA models in metabolomics studies. *Metabolomics* **2012**, *8*, 3–16. [[CrossRef](#)] [[PubMed](#)]
125. Worley, B.; Powers, R. Multivariate analysis in metabolomics. *Curr. Metabolomics* **2013**, *1*, 92–107. [[CrossRef](#)] [[PubMed](#)]
126. Gromski, P.S.; Muhamadali, H.; Ellis, D.I.; Xu, Y.; Correa, E.; Turner, M.L.; Goodacre, R. A tutorial review: Metabolomics and partial least squares-discriminant analysis: A marriage of convenience or a shotgun wedding. *Anal. Chim. Acta* **2015**, *879*, 10–23. [[CrossRef](#)] [[PubMed](#)]
127. Petré, H.; Köllner, T.G.; Junker, R.R. Quantifying chemodiversity considering biochemical and structural properties of compounds with the R package chemodiv. *New Phytol.* **2023**, *237*, 2478–2492. [[CrossRef](#)] [[PubMed](#)]
128. Benomari, F.Z.; Sarazin, M.; Chaib, D.; Pichette, A.; Boumghar, H.; Boumghar, Y.; Djabou, N. Chemical variability and chemotype concept of essential oils from Algerian wild plants. *Molecules* **2023**, *28*, 4439. [[CrossRef](#)] [[PubMed](#)]
129. Horváth, G.; Horváth, A.; Reichert, G.; Böszörményi, A.; Sipos, K.; Pandur, E. Three chemotypes of thyme (*Thymus vulgaris* L.) essential oil and their main compounds affect differently the IL-6 and TNF α cytokine secretions of BV-2 microglia by modulating the NF- κ B and C/EBP β signalling pathways. *BMC Complement. Med. Ther.* **2021**, *21*, 148. [[CrossRef](#)] [[PubMed](#)]
130. Gimenes, L.; Silva, J.C.R.L.; Facanali, R.; Hantao, L.W.; Siqueira, W.J.; Marques, M.O.M. Essential oils of new *Lippia alba* genotypes analyzed by flow-modulated comprehensive two-dimensional gas chromatography (GC $\times$ GC) and chemometric analysis. *Molecules* **2021**, *26*, 2332. [[CrossRef](#)] [[PubMed](#)]

131. Barros, L.S.P.; Santos da Cruz, E.N.; de Araújo Guimarães, B.; Setzer, W.N.; Veras Mourão, R.H.; do Rosário da Silva, J.K.; Silva da Costa, J.; Baia Figueiredo, P.L. Chemometric analysis of the seasonal variation in the essential oil composition and antioxidant activity of a new geraniol chemotype of *Lippia alba* (Mill.) N.E.Br. ex Britton & P. Wilson from the Brazilian Amazon. *Biochem. Syst. Ecol.* **2022**, *105*, 104503. [[CrossRef](#)]
132. Zinno, P.; Guantario, B.; Lombardi, G.; Ranaldi, G.; Finamore, A.; Allegra, S.; Mammano, M.M.; Fascella, G.; Raffo, A.; Roselli, M. Chemical composition and biological activities of essential oils from *Origanum vulgare* genotypes belonging to the carvacrol and thymol chemotypes. *Plants* **2023**, *12*, 1344. [[CrossRef](#)] [[PubMed](#)]
133. Gilles, M.; Antoniotti, S. Chemical and olfactory analysis of the volatile fraction of *Ocimum gratissimum* concrete from Madagascar. *Chem. Biodivers.* **2023**, *20*, e202300252. [[CrossRef](#)] [[PubMed](#)]
134. Rathore, S.; Mukhia, S.; Kapoor, S.; Bhatt, V.; Kumar, R.; Kumar, R. Seasonal variability in essential oil composition and biological activity of *Rosmarinus officinalis* L. accessions in the western Himalaya. *Sci. Rep.* **2022**, *12*, 3305. [[CrossRef](#)] [[PubMed](#)]
135. Patonay, K.; Szalontai, H.; Radácsi, P.; Zámoriné-Németh, É. Chemotypes and their stability in *Mentha longifolia* (L.) L.—A comprehensive study of five accessions. *Plants* **2021**, *10*, 2478. [[CrossRef](#)] [[PubMed](#)]
136. Segal, R.; Feuerstein, I.; Danin, A. Chemotypes of *Artemisia herba-alba* in Israel based on their sesquiterpene lactone and essential oil constitution. *Biochem. Syst. Ecol.* **1987**, *15*, 411–416. [[CrossRef](#)]
137. Wan, N.; Li, Y.; Huang, X.Y.; Li, Y.H.; Zheng, Q.; Wu, Z.F. A comparative evaluation of chemical composition and antimicrobial activities of essential oils extracted from different chemotypes of *Cinnamomum camphora* (L.) Presl. *Grasas Aceites* **2022**, *73*, e441. [[CrossRef](#)]
138. Elaissi, A.; Moumni, S.; Derbali, Y.; Khouja, M.; Abid, N.; Lynen, F.; Khouja, M.L. Chemical composition of essential oils of eight Tunisian *Eucalyptus* species and their antibacterial activity against strains responsible for otitis. *BMC Complement. Med. Ther.* **2021**, *21*, 209. [[CrossRef](#)] [[PubMed](#)]
139. Radwan, M.M.; Chandra, S.; Gul, S.; Elsohly, M.A. Cannabinoids, phenolics, terpenes and alkaloids of *Cannabis*. *Molecules* **2021**, *26*, 2774. [[CrossRef](#)] [[PubMed](#)]
140. Birenboim, M.; Chalupowicz, D.; Maurer, D.; Barel, S.; Chen, Y.; Fallik, E.; Paz-Kagan, T.; Rapaport, T.; Sadeh, A.; Kengisbuch, D.; et al. Multivariate classification of *Cannabis* chemovars based on their terpene and cannabinoid profiles. *Phytochemistry* **2022**, *200*, 113215. [[CrossRef](#)] [[PubMed](#)]
141. Carvalho, V.M.; de Almeida, F.G.; de Macêdo Vieira, A.C.; Rocha, E.D.; Cabral, L.M.; Strongin, R.M. Chemical profiling of *Cannabis* varieties cultivated for medical purposes in southeastern Brazil. *Forensic Sci. Int.* **2022**, *335*, 111309. [[CrossRef](#)] [[PubMed](#)]
142. Herwig, N.; Utgenannt, S.; Nickl, F.; Möbius, P.; Nowak, L.; Schulz, O.; Fischer, M. Classification of *Cannabis* strains based on their chemical fingerprint: A broad analysis of chemovars in the German market. *Cannabis Cannabinoid Res.* **2025**, *10*, 409–419. [[CrossRef](#)] [[PubMed](#)]
143. Salamone, S.; Walzl, L.; Pompignan, A.; Grassi, G.; Chianese, G.; Koeberle, A.; Pollastro, F. Phytochemical characterization of *Cannabis sativa* L. chemotype V reveals three new dihydrophenanthrenoids that favorably reprogram lipid mediator biosynthesis in macrophages. *Plants* **2022**, *11*, 2130. [[CrossRef](#)] [[PubMed](#)]
144. Vigil, J.M.; Stith, S.S.; Brockelman, F.; Keeling, K.; Hall, B. Systematic combinations of major cannabinoid and terpene contents in *Cannabis* flower and patient outcomes: A proof-of-concept assessment of the Vigil Index of *Cannabis* Chemovars. *J. Cannabis Res.* **2023**, *5*, 4. [[CrossRef](#)] [[PubMed](#)]
145. Lichman, B.R. The scaffold-forming steps of plant alkaloid biosynthesis. *Nat. Prod. Rep.* **2021**, *38*, 103–129. [[CrossRef](#)] [[PubMed](#)]
146. Veeramohan, R.; Zamani, A.I.; Azizan, K.A.; Goh, H.-H.; Aizat, W.M.; Razak, M.F.A.; Yusof, N.S.M.; Mansor, S.M.; Baharum, S.N.; Ng, C.L. Comparative metabolomics analysis reveals alkaloid repertoires in young and mature *Mitragyna speciosa* (Korth.) Havil. leaves. *PLoS ONE* **2023**, *18*, e0283147. [[CrossRef](#)] [[PubMed](#)]
147. Damiani, T.; Smith, J.; Hebra, T.; Perković, M.; Čičak, M.; Kadlecová, A.; Rybka, V.; Dračínský, M.; Pluskal, T. Computational metabolomics reveals overlooked chemodiversity of alkaloid scaffolds in *Piper fimbriatum*. *Plant J.* **2025**, *121*, e70086. [[CrossRef](#)] [[PubMed](#)]
148. Meng, Y.; Du, Q.; Du, H.; Wang, Q.; Wang, L.; Du, L.; Liu, P. Analysis of chemotypes and their markers in leaves of core collections of *Eucommia ulmoides* using metabolomics. *Front. Plant Sci.* **2023**, *13*, 1029907. [[CrossRef](#)] [[PubMed](#)]
149. Missinou, A.A.; Ferreira de Carvalho, J.; Marnet, N.; Delhay, T.; Hamzaoui, O.; Abdel Sayed, D.; Guittou, Y.; Lebreton, L.; Langrume, C.; Laperche, A.; et al. Identification and quantification of glucosinolates and phenolics in a large panel of *Brassica napus* highlight valuable genetic resources for chemical ecology and breeding. *J. Agric. Food Chem.* **2022**, *70*, 5245–5261. [[CrossRef](#)] [[PubMed](#)]
150. Gohain, B.; Kumar, P.; Malhotra, B.; Bisht, N.C. A comprehensive Vis-NIRS equation for rapid quantification of seed glucosinolate content and composition across diverse *Brassica* oilseed chemotypes. *Food Chem.* **2021**, *354*, 129527. [[CrossRef](#)] [[PubMed](#)]

151. Katz, E.; Li, J.-J.; Jaegle, B.; Ashkenazy, H.; Abrahams, S.R.; Bagaza, C.; Holden, S.; Pires, C.J.; Angelovici, R.; Kliebenstein, D.J. Genetic variation, environment and demography intersect to shape Arabidopsis defense metabolite variation across Europe. *eLife* **2021**, *10*, e67784. [[CrossRef](#)] [[PubMed](#)]
152. Unger, K.; Raza, S.A.K.; Mayer, T.; Reichelt, M.; Stuttmann, J.; Hielscher, A.; Wittstock, U.; Gershenzon, J.; Agler, M.T. Glucosinolate structural diversity shapes recruitment of a metabolic network of leaf-associated bacteria. *Nat. Commun.* **2024**, *15*, 8496. [[CrossRef](#)] [[PubMed](#)]
153. Kazemi-Dinan, A.; Sauer, J.; Stein, R.J.; Krämer, U.; Müller, C. Is there a trade-off between glucosinolate-based organic and inorganic defences in a metal hyperaccumulator in the field? *Oecologia* **2015**, *178*, 369–378. [[CrossRef](#)] [[PubMed](#)]
154. Van Leur, H.; Raaijmakers, C.E.; van Dam, N.M. A heritable glucosinolate polymorphism within natural populations of *Barbarea vulgaris*. *Phytochemistry* **2006**, *67*, 1214–1223. [[CrossRef](#)] [[PubMed](#)]
155. Tewes, L.J.; Müller, C. Interactions of *Bunias orientalis* plant chemotypes and fungal pathogens with different host specificity in vivo and in vitro. *Sci. Rep.* **2020**, *10*, 10750. [[CrossRef](#)] [[PubMed](#)]
156. Le Boulch, P.; Missinou, A.A.; Boutet, S.; Perreau, F.; Manzanares-Dauleux, M.J.; Falentin, C.; Filangi, O.; Lariagon, C.; Bouchereau, A.; Rousseau-Gueutin, M.; et al. Metabolomic analysis of a core collection of *Brassica rapa* and *Brassica oleracea* unveils unexpected chemical diversity with potential applications in chemical ecology and breeding. *BMC Plant Biol.* **2026**, *26*, 569. [[CrossRef](#)] [[PubMed](#)]
157. Da Costa-Oliveira, C.; Gouvêa-Silva, J.G.; Dos Santos Fernandes Muniz, R.; Felisberto, J.S.; Mazzola, P.G.; Pereira, R.C.; Moreira, D.L.; Ramos, Y.J. *Cannabis*: From crop to shop—Some insights about stability to access quality control. *J. Cannabis Res.* **2026**, *8*, 45. [[CrossRef](#)] [[PubMed](#)]
158. Sasidharan, R.; Grond, S.G.; Champion, S.; Eilers, E.J.; Müller, C. Intraspecific plant chemodiversity at the individual and plot levels influences flower visitor groups with consequences for germination success. *Funct. Ecol.* **2024**, *38*, 2665–2678. [[CrossRef](#)]
159. Ojeda-Prieto, L.; Medina-van Berkum, P.; Unsicker, S.B.; Heinen, R.; Weisser, W.W. Intraspecific chemical variation of *Tanacetum vulgare* affects plant growth and reproductive traits in field plant communities. *Plant Biol.* **2025**, *27*, 785–801. [[CrossRef](#)] [[PubMed](#)]
160. Thon, F.M.; Müller, C.; Wittmann, M.J. The evolution of chemodiversity in plants: From verbal to quantitative models. *Ecol. Lett.* **2024**, *27*, e14365. [[CrossRef](#)] [[PubMed](#)]
161. Wittmann, M.J.; Bräutigam, A. How does plant chemodiversity evolve? Testing five hypotheses in one population genetic model. *New Phytol.* **2025**, *245*, 1302–1314. [[CrossRef](#)] [[PubMed](#)]
162. Müller, C.; Fuchs, B.; Schnitzler, J.-P.; Unsicker, S.B.; Whitehead, S.R. Ecology and evolution of plant chemodiversity. *Plant Biol.* **2025**, *27*, 633–636. [[CrossRef](#)] [[PubMed](#)]
163. Heywood, V.H. The conservation of genetic and chemical diversity in medicinal and aromatic plants. In *Biodiversity: Biomolecular Aspects of Biodiversity and Innovative Utilization*; Şener, B., Ed.; Springer: Boston, MA, USA, 2002; pp. 13–22. [[CrossRef](#)]
164. Xiao, Q.; Mu, X.; Liu, J.; Li, B.; Liu, H.; Zhang, B.; Xiao, P. Plant metabolomics: A new strategy and tool for quality control of Chinese medicinal materials. *Chin. Med.* **2022**, *17*, 46. [[CrossRef](#)] [[PubMed](#)]
165. Wang, H.; Chen, Y.; Wang, L.; Liu, Q.; Yang, S.; Wang, C. Advancing herbal medicine: Enhancing product quality and safety through robust quality control practices. *Front. Pharmacol.* **2023**, *14*, 1265178. [[CrossRef](#)] [[PubMed](#)]
166. Ji, P.; Yang, X.; Zhao, X. Application of metabolomics in quality control of traditional Chinese medicine: A review. *Front. Plant Sci.* **2024**, *19*, 1463666. [[CrossRef](#)] [[PubMed](#)]
167. De Sousa, D.P.; Damasceno, R.O.S.; Amorati, R.; Elshabrawy, H.A.; de Castro, R.D.; Bezerra, D.P.; Nunes, V.R.V.; Gomes, R.C.; Lima, T.C. Essential oils: Chemistry and pharmacological activities. *Biomolecules* **2023**, *13*, 1144. [[CrossRef](#)] [[PubMed](#)]
168. Jităreanu, A.; Trifan, A.; Vieriu, M.; Caba, I.-C.; Mărţu, I.; Agoroaei, L. Current trends in toxicity assessment of herbal medicines: A narrative review. *Processes* **2022**, *11*, 83. [[CrossRef](#)]
169. Soares, C.H.N.; Damasceno Junior, P.C.; Campos, I.M.; Amorim, G.T.S.; Carmo, M.G.F.; Chaves, D.S.A.; Souza, M.A.A. Selection of genotypes (citral chemotype) of *Lippia alba* (Mill.) N. E. Brown regarding seasonal stability of the essential oils chemical profile. *Ind. Crops Prod.* **2019**, *139*, 111497. [[CrossRef](#)]
170. Fernandez, O.; Millet, E.J.; Rincet, R.; Prigent, S.; Pétriacq, P.; Gibon, Y. Plant metabolomics and breeding. *Adv. Bot. Res.* **2021**, *98*, 207–235. [[CrossRef](#)]
171. Brzozowski, L.J.; Campbell, M.T.; Hu, H.; Caffè-Treml, M.; Gutiérrez, L.; Smith, K.P.; Sorrells, M.E.; Gore, M.A.; Jannink, J.-L. Generalizable approaches for genomic prediction of metabolites in plants. *Plant Genome* **2022**, *15*, e20205. [[CrossRef](#)] [[PubMed](#)]
172. Pacheco-Ruiz, P.; Postacchini, S.; Mazzoni, L.; Vallarino, J.G. Metabolomics for plant breeding: Knowledge without adoption? *Front. Plant Sci.* **2026**, *17*, 1832662. [[CrossRef](#)] [[PubMed](#)]
173. Lynch, R.C.; Padgitt-Cobb, L.K.; Garfinkel, A.R.; Knaus, B.J.; Hartwick, N.T.; Allsing, N.; Aylward, A.; Bentz, P.C.; Carey, S.B.; Mamerto, A.; et al. Domesticated cannabinoid synthases amid a wild mosaic of *Cannabis* pangenome variation. *Nature* **2025**, *643*, 1001–1010. [[CrossRef](#)] [[PubMed](#)]

174. Hawkins, C.; Ginzburg, D.; Zhao, K.; Dwyer, W.; Xue, B.; Xu, A.; Rice, S.; Cole, B.; Paley, S.; Karp, P.; et al. Plant Metabolic Network 15: A resource of genome-wide metabolism databases for 126 plants and algae. *J. Integr. Plant Biol.* **2021**, *63*, 1888–1905. [[CrossRef](#)] [[PubMed](#)]
175. Tian, Z.; Hu, X.; Xu, Y.; Liu, M.; Liu, H.; Li, D.; Hu, L.; Wei, G.; Chen, W. PMhub 1.0: A comprehensive plant metabolome database. *Nucleic Acids Res.* **2024**, *52*, D1579–D1587. [[CrossRef](#)] [[PubMed](#)]
176. Wang, P.; Moore, B.M.; Calderon, A.I.; Weng, J.K. Computational prediction of plant metabolic pathways. *Curr. Opin. Plant Biol.* **2022**, *66*, 102170. [[CrossRef](#)] [[PubMed](#)]
177. Bai, W.; Li, C.; Li, W.; Wang, H.; Han, X.; Wang, P.; Wang, L. Machine learning assists prediction of genes responsible for plant specialized metabolite biosynthesis by integrating multi-omics data. *BMC Genom.* **2024**, *25*, 418. [[CrossRef](#)] [[PubMed](#)]
178. Durand, M.; Besseau, S.; Papon, N.; Courdavault, V. Unlocking plant bioactive pathways: Omics data harnessing and machine learning assisting. *Curr. Opin. Biotechnol.* **2024**, *87*, 103135. [[CrossRef](#)] [[PubMed](#)]
179. Hu, Y.; Ruan, Y.; Zhao, X.-L.; Jiang, F.; Liu, D.; Zhu, Q.; Zhang, Q.-Y.; Yang, Q.-Y. PCMD: A multilevel comparison database of intra-and cross-species metabolic profiling in 530 plant species. *Plant Commun.* **2024**, *5*, 101069. [[CrossRef](#)] [[PubMed](#)]
180. Danilevicz, M.F.; Gill, M.; Anderson, R.; Batley, J.; Bennamoun, M.; Bayer, P.E.; Edwards, D. Plant genotype to phenotype prediction using machine learning. *Front. Genet.* **2022**, *13*, 822173. [[CrossRef](#)] [[PubMed](#)]
181. Chi, J.; Shu, J.; Li, M.; Mudappathi, R.; Jin, Y.; Lewis, F.; Boon, A.; Qin, X.; Liu, L.; Gu, H. Artificial intelligence in metabolomics: A current review. *TrAC Trends Anal. Chem.* **2024**, *178*, 117852. [[CrossRef](#)] [[PubMed](#)]
182. Mansoor, S.; Karunathilake, E.M.B.M.; Tuan, T.T.; Chung, Y.S. Genomics, phenomics, and machine learning in crop improvement. *Hortic. Plant J.* **2025**, *11*, 2. [[CrossRef](#)]
183. Kim, H.W.; Wang, M.; Leber, C.A.; Nothias, L.-F.; Reher, R.; Kang, K.B.; van der Hooft, J.J.J.; Dorrestein, P.C.; Gerwick, W.H.; Cottrell, G.W. NPClassifier: A deep neural network-based structural classification tool for natural products. *J. Nat. Prod.* **2021**, *84*, 2795–2807. [[CrossRef](#)] [[PubMed](#)]
184. Prete, A.L.; Corradini, B.T.; Costanti, F.; Scarselli, F.; Bianchini, M. Leveraging molecular graphs for natural product classification: Opportunities for graph neural networks. *Comput. Struct. Biotechnol. J.* **2025**, *27*, 3837–3884. [[CrossRef](#)] [[PubMed](#)]
185. Chen, J.; Cai, J.; Duong, H.T.Q.; Bunsupa, S.; Han, R.; Tong, X. AI-driven integration and optimization of medicinal plant multi-omics metabolic networks. *Front. Plant Sci.* **2026**, *17*, 1756809. [[CrossRef](#)] [[PubMed](#)]
186. Wang, M.; Carver, J.J.; Phelan, V.V.; Sanchez, L.M.; Garg, N.; Peng, Y.; Nguyen, D.D.; Watrous, J.D.; Kapon, C.A.; Luzzatto-Knaan, T.; et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking. *Nat. Biotechnol.* **2016**, *34*, 828–837. [[CrossRef](#)] [[PubMed](#)]
187. Sud, M.; Fahy, E.; Cotter, D.; Azam, K.; Vadivelu, I.; Burant, C.; Edison, A.; Fiehn, O.; Higashi, R.; Nair, K.S.; et al. Metabolomics Workbench: An international repository for metabolomics data and metadata, metabolite standards, protocols, tutorials and training, and analysis tools. *Nucleic Acids Res.* **2016**, *44*, D463–D470. [[CrossRef](#)] [[PubMed](#)]
188. Nothias, L.F.; Petras, D.; Schmid, R.; Dührkop, K.; Rainer, J.; Sarvepalli, A.; Protasyuk, I.; Ernst, M.; Tsugawa, H.; Fleischauer, M.; et al. Feature-based molecular networking in the GNPS analysis environment. *Nat. Methods* **2020**, *17*, 905–908. [[CrossRef](#)] [[PubMed](#)]
189. Aron, A.T.; Gentry, E.C.; McPhail, K.L.; Nothias, L.-F.; Nothias-Esposito, M.; Bouslimani, A.; Petras, D.; Gauglitz, J.M.; Sikora, N.; Vargas, F.; et al. Reproducible molecular networking of untargeted mass spectrometry data using GNPS. *Nat. Protoc.* **2020**, *15*, 1954–1991. [[CrossRef](#)] [[PubMed](#)]
190. Yurekten, O.; Payne, T.; Tejera, N.; Amaladoss, F.X.; Martin, C.; Williams, M.; O'Donovan, C. MetaboLights: Open data repository for metabolomics. *Nucleic Acids Res.* **2024**, *52*, D640–D646. [[CrossRef](#)] [[PubMed](#)]
191. Bremer, P.L.; Fiehn, O. SMetaS: A sample metadata standardizer for metabolomics. *Metabolites* **2023**, *13*, 941. [[CrossRef](#)] [[PubMed](#)]
192. Karakas, E.; Rai, A.; Singh, S. The use of web resources for metabolomics in horticultural crops. *Hortic. Adv.* **2025**, *3*, 18. [[CrossRef](#)]
193. Xavier, J.K.A.M.; Baia, T.G.C.; Alegria, O.V.C.; Figueiredo, P.L.B.; Carneiro, A.R.; Moreira, E.C.d.O.; Maia, J.G.S.; Setzer, W.N.; da Silva, J.K.R. Essential Oil Chemotypes and Genetic Variability of *Cinnamomum verum* Leaf Samples Commercialized and Cultivated in the Amazon. *Molecules* **2022**, *27*, 7337. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.